

Too many official secrets

Any of the many thousands of scientists and engineers employed by the British government, either directly or indirectly, would be guilty of a misdemeanour if he or she communicated a "code word, pass word, sketch, plan, model, article, note, document or information to any person other than a person to whom he is authorised to communicate it, or a person to whom it is, in the interest of the State, his duty to communicate it". So states Section 2 of the Official Secrets Act and to press the point home posters at workplaces declare "You must not talk about or pass on information about your work unless you are authorised to do so", which is a more restricted reading of the meaning of the act. But every day scientists do just that in the course of communicating with the outside world, whilst others unsure about their position *vis-à-vis* the act are genuinely inhibited from talking about the implications of their work, criticising government policy in any way, writing to the press to correct genuine errors and so on. The Official Secrets Act is occasionally looked at (as, for instance, by the Franks Commission in 1971-2) and there are now and then promises of a White Paper proposing new legislation (one is due at the moment, but no delivery date is mentioned). Meanwhile, a law is variously ignored and feared; this cannot be for the common good.

Few would deny that as far as Britain's defence research goes, those who have chosen to work in the field are probably ill-advised to start delivering pass-words, models and so on to the military attachés of certain embassies in London. And whilst clearance of scientific manuscripts for publication is an irksome business, involving people who know little or nothing of the content, there are few complaints about the unreasonable withholding of approval. It is only when one moves into the more informal channels of communication that the ground becomes more shaky. If an employee of Harwell who is a parent is asked to hand out the prizes after Sports Day and make a few encouraging remarks to the assembled company, should those remarks be cleared? If a scientist spots a technical error or a misunderstanding in a press report on a sub-

ject in which he is generally competent, should approval be sought before any approach is made to the media to make a correction? If it seems that incompetence in some part of the civil service is severely holding up progress on some project which is generally regarded as desirable, does a potential whistle-blower have to clear any remarks he may wish to make with the very authorities whom he wishes to criticise? The answer to all these questions is yes, and accounts for the almost invisibility of many civil servants—not perhaps a severe loss to sports days, but certainly a severe impediment to public debate. Of course, many scientists working for the government are very helpful indeed, willing to answer questions directly, willing to talk freely about their work and its implications. But few can afford to be seen to be doing so when the issue at stake is at all controversial.

One of the problems is the extraordinary generality of the word 'information'. Anything from how to make a nuclear device to the identity of the tea-lady comes within this category, and perhaps most troubling, 'information' need not simply be basic facts but could be interpreted as almost any general understanding of the way things are that accrues after working anywhere for a period of time. Likewise there is ambiguity in the concession that information can be transferred, unauthorised, "if it is in the interest of the State". If the boss is incompetent and no-one within the organisation will listen, the state's interest is probably served by telling all, as a last resort.

Most people who work for large organisations get used to 'the system'—an almost totally unwritten code of what should and shouldn't be done, what competitors may not be told, how much the organisation should be criticised, how much defended. Organisations do not suffer badly from leaving their employees to work out from common sense what the system is. Civil servants also work within a system, applying common sense much of the time in their external relations. But over it all hovers an ambiguous act which can and often does over-rule common sense. The time has surely come to cut back on the act's scope. □



Solar energy: bonanza or bunfight?

David Dickson describes the background to President Carter's announcement of a major boost to the US solar energy effort

ONE of the biggest mistakes that President Jimmy Carter has made in 1978 has turned out to be a relatively small item in the proposed 1979 budget for the Department of Energy submitted to Congress in January. This was the suggestion that the amount of money allocated to research and development of solar technologies should be reduced from \$411 million in 1978 to \$400 million in 1979.

At the time the administration justified this reduction on the grounds that many solar developments had reached the stage at which they could be successfully taken up by private industry. Later, department officials added that they had expected the solar R & D budget to be substantially increased by Congress anyway, as had happened in previous years.

But the damage had been done. Environmentalists, concerned at the president's equivocal stand on nuclear energy, pointed to the reduced solar budget as a clear betrayal of campaign promises. And their message spread. An opinion poll recently found that 80% of those interviewed felt the government should be doing more research into solar technologies (compared to less than half for nuclear technologies). And the reduction of the solar R & D budget is now widely quoted among those who had previously been Carter supporters as a reason for their disillusionment with his performance.

The statement which Carter was due to give yesterday as part of the international 'Sun-day' activities—and symbolically presented at the site of the new Solar Energy Research Institute near Denver in Colorado—therefore had a dual purpose. Not merely was the president keen to correct the impression that his administration is anti-solar, he also had one eye firmly on Edmund (Jerry) Brown, Governor of California and a potential rival for the 1980 presidential nomination, who is leading his state towards a solar future.

In his Sun-day speech Carter was expected to reflect the growing enthusiasm for solar energy among the American people, for many of whom the promise of a clean and renewable energy resource lacking the waste problems of nuclear or fossil fuels now exerts a strong, almost mystical, attraction. This enthusiasm is itself reflected in the activities of two separate Washington-based coalitions both of which embrace a range of political positions and interests in focusing on the promotion of solar energy.

Sun-day itself has been organised through a wide range of groups sharing interests in environmental matters, and is largely the brainchild of Denis Hayes, organiser of the highly-successful 'Earth day' in 1970 which played an important part in injecting the word ecology into the national vocabulary. The second grouping, less publicised but equally influential, is known as the 'solar coalition', a movement that started primarily among congressional aides and has put together a package of energy bills which, as the result of rapidly increasing interest in recent months, is now claimed to have the support of almost a quarter of the members of Congress.

While both of these coalitions have been applying external pressure within the administration itself the main push for a strong initiative on solar energy has come from the Council on Environmental Quality. Drawing on a number of sources and analyses, such as Amory Lovins's *Soft Energy Paths* and Denis Hayes's book *Rays of Hope*, the CEQ has recently produced a report in which it claims that, given sufficient national effort, the US could meet up to one quarter of its energy needs through solar technology by 2000.

Largely at the initiative of the CEQ the president's domestic council subsequently drew up a proposal for a new solar strategy, including both short-term projects and a full-scale review of federal activities in the solar area. These formed the basis for the proposals which the president was expected to present yesterday.

A political issue

But given that the administration now seems prepared to make an extra commitment—though not as large as some would like—to solar energy, the central question is now not whether the development of solar technologies will take place, but in what direction. As the president's manoeuvrings indicate, the whole issue of solar energy has become overtly political, and the questions it begs are no longer merely technical, but economic and social as well. And it is the shifting balance between different types of judgement that have given rise to deep splits within government bodies on how a solar development programme should be pursued.

Until a few years ago, for example, the Department of Energy had shown a general scepticism towards the potential of solar energy. More recently this has given way to the realisation that solar



Consolidated Edison's windmill on top of a tenement building in New York

energy could become a viable addendum to a nuclear programme. Initially, however, DOE programmes were developed according to the same philosophy as had been used for fossil and nuclear fuels, namely that the most efficient and effective power production involved large centralised facilities, and that a development programme should head towards these by building up from small-scale demonstration plants. Two examples of this which gained early support have been the power-tower concept, which involves a large array of mirrors all reflecting solar rays on to a central receiver, and the scheme for obtaining energy from the thermal gradient in the oceans (OTEC).

Further shifts have subsequently taken place, partly under pressure from Congress, leading the department to take an increasingly active interest in areas such as photovoltaics, windpower and biomass. And the greater divergence these have involved from the nuclear power development philosophy, the greater have been the organisational strains as the department has attempted to adapt.

Similar problems are being experienced by the utility companies charged with the responsibility of delivering power to consumers. Many of these, like the DOE, were initially highly sceptical of solar energy. And as they have been brought round to accepting it, the tendency has been to concentrate on centralised, capital-intensive facilities. Two concerns in particular have been voiced by the utility companies. The first is that the transition to solar should proceed in an orderly manner, taking into account existing systems and modes of operation. The second

concern is with utility rates. Many utilities, for example, argue that the need to continue to provide back-up facilities for those times when solar energy is not available will not merely mean no reduction in electricity prices, but could even increase them by distorting the load patterns for which existing rates have been developed. Related to this is the fear that a decentralised power source could threaten the position of the utility companies. In a recent Manhattan court case the utility company Consolidated Edison was ordered by the New York public service commission to buy back the extra power generated on particularly windy days by a large windmill constructed on the roof of a renovated tenement building.

New solar institutions

Another institution that has found itself having to adapt to the political circumstances surrounding the rise to popularity of solar energy is the new Solar Energy Research Institute (SERI) at Golden near Denver.

The proposal for such an institute was first made in legislation passed in the aftermath of the Arab oil embargo of 1973. After the idea of a single centre, relatively protected from political pressure, had been vetted and approved by the National Academy of Sciences, a competition was held to find the most suitable site.

The competition was won by a proposal from the Midwest Research Institute, a non-profit organisation which had prepared a plan based on the Golden site. However when the winner was announced, the administration said that it intended to establish four additional regional research institutes. These had never been contemplated either by Congress or by the NAS, and the fact that their siting reflected the constituency interests of key Democrat congressmen, such as Hubert Humphrey and Edward Kennedy, only reinforced concern that the political pork-barrel was at work.

After considerable dispute, and an internal inquiry under the auspices of John Deutch, head of the Office of Energy Research, it was finally agreed last month that the four regional institutes will still be developed, but will have a different role from SERI. The latter will remain the prime institution with regard to R & D; the regional institutions will be more concerned with pushing proven solar technology out into the market place. SERI has now, albeit reluctantly, accepted this compromise. But it is also having to live closer to the DOE than it would have liked, being told what to do rather than determine its own strategy.

The director of SERI, for example, Dr Paul Rappoport, says that he is

relatively happy with the present arrangements. However he does not deny that he would prefer to be working on a longer timescale than the 1985 perspective for which Energy Secretary Dr James Schlesinger is aiming.

The DOE, the utility companies, and SERI are all therefore having to cope with demands that do not grow directly out of the logic of their previous paths. And unlike countries such as the United Kingdom, in which Energy Secretary Tony Benn has consciously designed his energy strategy as an agent of social policy, what is emerging in the US is a joint strategy whose aim is to reinforce, rather than replace, the market system as the basic mechanism for the distribution of energy.

In the DOE for example, 'commercialisation' has become a key word used by research planners. Thus an internal memorandum to Secretary Schlesinger suggests a strategy for bringing down the costs of photovoltaic cells by linking R & D efforts to a large scale commercial exercise which would, for a period of years, guarantee to meet the difference between the costs at which cells can be produced and the price which users are prepared to pay. At SERI, too, much of the emphasis is on finding ways of moving solar technology to the point where its development can be left to the dynamics of market pull.

Environmentalists fears

But what concerns environmentalists and others is that the increasing presence of large corporate interests in the field of solar energy could seriously distort the development of the technology away from the socially desirable towards the merely profitable. As an example, they point to plans that are rapidly gathering support in Congress to build vast solar satellites each capable of producing enough power to meet the needs of an average city. A bill proposing a feasibility study

for such systems has already been introduced into the House, largely as a result of pressure from a group known as the Sunsat Energy Council, whose members include companies such as Boeing, McDonnell Douglas, Lockheed Corporation and General Electric.

Many of these firms are also closely involved in the development of large land-based solar systems. Others are concerned that utility companies are pushing for centralised power systems, rather than attempting to exploit the full potential for decentralised power production that solar energy offers.

The contrast between idealism and political realities is everywhere apparent. Thus the organisers of Sunday claim that the widespread use of solar systems "fits well into a political system that emphasises decentralisation and local control". In contrast, the president of an association of 1,300 small fuel oil producers in New England who sell and maintain solar panels admits: "we see that solar is coming, and we want to control as much of the market as we can".

Even on costs, slogans are deceptive. Great play is made of the fact that sunshine is free; yet as scientists at SERI point out, its effective conversion is far from free, and is in fact capital-intensive. Its economic advantages over other existing fuels can only be demonstrated by inserting other costs—such as environmental and health damage—into planning equations which few economists can yet effectively do.

Such contradictions are coming home to roost in the White House itself. Last month the president's cousin, Hugh Carter, who is largely responsible for the White House budget, complained that a plan submitted by the DOE to provide hot water by solar power was "not economically justifiable at today's fuel prices." What the president will do about his own domestic arrangements is awaited with interest. □

Collecting solar energy in the desert



Trinidad wants more autonomy for University of the West Indies' campuses



University buildings at St. Augustine, Trinidad

THE government of Trinidad and Tobago has recently published a White Paper on science and technology which has raised something of a storm in the Caribbean. Many see it as a blow to the regional University of the West Indies. It suggests that Trinidad and Tobago should set up a National Institute of Higher Education (Research, Science and Technology) because the university is unsuited to their needs.

The university is plagued by the administrative problems of running three separate campuses one in each of Trinidad, Barbados and Jamaica (Trinidad and Jamaica are 1,000 miles apart). Now, however, the oil age has added an extra dimension to its problems. Trinidad and Tobago are riding high on oil, but the other economies, especially Jamaica's, are in trouble. Consequently

Trinidad and Tobago feel they are being hampered in their industrialisation programme by a bureaucratic and unadaptable university.

This is not new: in the late 1960s similar charges came from Jamaica when its economy was buoyant. The charge of unwieldiness in the system is impossible to refute: decisions creep through a tangle of committees in a structure which some staff on the smaller campuses view as a way to retain power. There are also problems of any multi-national university: inadequate financing, governments in arrears and conflicting national objectives.

The latest proposals from Trinidad and Tobago seek to give more autonomy to the individual campuses. The White Paper outlines possible new management and financing structures for

the university. It suggests that the cost of each campus should be borne by the 'host' government and that a small central university authority should have an advisory role over finance and new developments. (The central authority would maintain control of planning of regional programmes and execution of common university services.)

The university response to these comments has been to admit to bureaucratic inefficiency. But it also points out that it has worked on at least nine government financed projects including several funded by Trinidad and Tobago. It also points to agreed expansion in engineering (especially in petroleum engineering) and to the planned medical school in Trinidad.

Outside the university is the feeling that an institution of 800 academics is bound to be expensive for some of the territories, and even in the best circumstances university research is unlikely to have the kind of dramatic impact which impatient developing countries want. Inside the university there is the feeling that bureaucracy has brought this action on itself. But there is another view: that while both comments above are quite correct, the White Paper is a political ploy. At one level the paper seeks to convey an image of a go-ahead government restrained by a slow-motion university; at another it creates the impression of a dynamic economy held back by a number of troubled ones.

There is at least one clear reference to the effect of differing ideologies. Jamaica and Guyana have, for some years, proclaimed themselves socialist states of one kind or another. One effect of this was a call for 'democratisation' of the campuses and 'involvement of all in the decision making process'. The White Paper proposes a return to the autocratic structure on the Trinidad campus: "Thus the so-called democratisation process may develop on the Jamaica campus independent of whatever management structure evolves on the Trinidad and Tobago campus".

Philbert Morris

What the White Paper says

Science in Trinidad has been "influenced on the one hand by the establishment of the University of the West Indies and on the other, by the absence of any meaningful indigenous activities, within the private sector.... Substantial resources were channelled into the university, with the hope that its efforts in the medical sciences, engineering, education and management would be adequate to meet the demands of the country. The industrial sector, dominated by the trans-national corporations, relied exclusively on imported technology for its own needs. Such foreign knowledge was applied without being absorbed by the internal technological infrastructure".

The paper talks of the:

- "absence of a policy for technology related to national objectives;
- a complete lack of coordination of the national effort in technology;
- the growing tendency towards individual effort of both persons and organisations;
- the proliferation of new institutions,

new advisory groups, councils, committees, etc;

- lack of relationship between the education plan and national needs;
- lack of a coherent plan for education oriented towards technology;
- absence of an environment that places science and technology in its proper perspective;
- a continuing and increasing dependence on imported technology with all the worst features of the traffic in technology;
- limited initiatives in research and development;
- complete neglect by local business of research and development; it hardly ever appears as a legitimate cost in local operations;
- the brain drain;
- an almost static picture in the level of technology;
- a growing deterioration in the level of technology in certain areas—for example road maintenance and production of certain agricultural crops—in which there was originally a reasonable level in indigenous technology."

NIH confirms violation of recombinant DNA research guidelines

AN internal inquiry conducted by the National Institutes of Health has confirmed allegations first made public last December that a research worker at Harvard Medical School carried out experiments involving recombinant DNA techniques in violation of guidelines laid down by the NIH.

The inquiry has revealed that Dr Charles A. Thomas, then professor of biological chemistry and now with the Scripps Clinic and Research Foundation at La Jolla, California, carried out research using such techniques without filing a 'memorandum of understanding and agreement' as required by the NIH guidelines published in 1976.

It also found that although the Harvard Medical School Committee on Recombinant DNA Molecules had, for a number of reasons, not approved an MUA submitted by Dr Thomas in November 1976, he had continued to do recombinant DNA research up to December 1977 without the committee knowing.

The NIH inquiry was carried out by Mr James Schriver, director of the institutes' division of management and review, at the request of NIH director Dr Donald Fredrickson. The allegations

that Dr Thomas had failed to file an MUA were made by an environmentalist group following information received through a 'freedom of information' petition.

In his report Mr Schriver says that although no mention was made by Dr Thomas in various grant applications to the NIH that experiments involving DNA molecules were to be performed, a grant from NIH was cited as supporting a paper published in May 1976 which described DNA cloning.

Mr Schriver reports that at a meeting last December, Dr Thomas stated that he had been performing recombinant DNA experiments continuously at the P2 level. "He made this statement even though virtually all the support he had been receiving for his research consisted of his NIH grants which did not contemplate experiments of this type," Mr Schriver says.

The report also describes lengthy negotiations which took place between Dr Thomas and the medical school's Recombinant DNA Executive Committee about whether Dr Thomas' research facilities met the containment requirements laid down in the NIH guidelines for the type of research that

he was carrying out. In particular, Dr Thomas had initially argued that his laboratory met the requirements for P3 containment levels: the resultant dispute with the Harvard Committee contributed to the delays in filing a MUA.

Harvard Medical School recently released its own report on the affair, prepared by an *ad hoc* university faculty committee. It says that the committee had been convinced that the research directed by Dr Thomas had been carried out safely with no threat to the health of those involved.

It says that the failure to file a MUA with the NIH was the combined result of misunderstandings and differences of opinion within Harvard about the requirements embodied in the NIH guidelines, failures in communication between Dr Thomas, the Recombinant DNA Executive Committee and the NIH, and administrative lapses in the latter two institutions. Guilt did not rest with one person alone, it added.

Dr Fredrickson said last week that he had asked the NIH Executive Recombinant DNA Committee to analyse both reports and make recommendations about what action would be taken.

David Dickson

WMO planning world climate study

A MEETING hosted earlier this year by the International Institute of Applied Systems Analysis (IIASA) on behalf of the World Meteorological Organisation (WMO) set the seal on preparations for the World Climate Conference, to be held in Geneva in February next year. The conference will be an essential preliminary to the World Climate Programme, a massive project in which virtually all the specialised agencies of the UN system will be involved in one way or another, with WMO as the "lead agency".

We really know remarkably little about climate. The big, WMO-sponsored projects such as the 'global atmosphere research programme' (GARP) and the 'world weather watch' are now beginning to show some results, and these will be among the inputs to next year's conference, together with results from various inter-agency projects in which WMO is also involved. But the main objective will be to assess our present knowledge of climate, and to plan a long term, global research programme. Such a programme, WMO points out, must be based on accurate, readily available data, including past records of whatever sort; forecasting, in particular for longer ranges than is at present possible; and hence, studies of the impact of climate on human

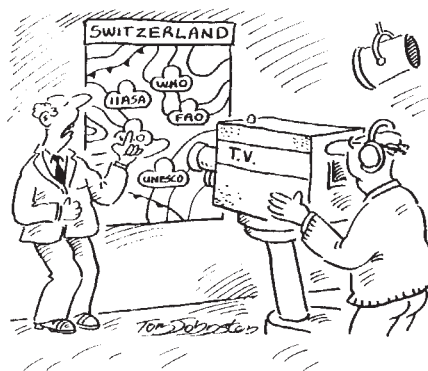
activities, and the feed-back from those activities, such as the possible effects on the atmosphere of increasing amounts of CO₂.

The recent meeting was a preliminary gathering of 20 to 25 meteorologists and others who were invited to contribute major papers to the conference itself. The idea was to avoid duplication and unnecessary overlapping at the main meeting. Each of the specialised agencies involved—UNESCO, FAO, ICAO for example—contributed papers in their own special fields, while IIASA was concerned in

the production and operation of computer models, now being increasingly used in climate studies. The main conference, which has a multi-agency planning committee, will last two weeks. In sharp contrast to such recent UN gatherings as those concerned with water and desertification, it will be a strictly scientific affair, with probably not much over 100 participants. After consideration by the next World Meteorological Congress, due in the Spring of 1979, the conclusions of the conference should form a basis for planning the World Climate Programme itself. This in turn will be the subject of a major planning and administrative conference some months later.

The quiet way in which WMO is going about this whole project is typical of this small, but perhaps most efficient of UN specialised agencies. In particular, the insistence that the recent meeting was scientific should have avoided the previous publicity and subsequent disillusion associated with too many large conferences within the UN system. The conclusions drawn and activities planned should form a part of WMO's own submission to the next of these events, the UN Conference on Science and Technology for Development, to be held in Vienna next year.

Peter Collins



"There will be heavy showers of acronyms in the Geneva area!"

West Germany spends more on coal research than on fast breeder research

WEST GERMANY produces 190 million tonnes of hard coal and brown coal a year, and reserves are so great that, at this rate, it is estimated there will be enough to last 400 years. One of the most pressing problems for German energy policy therefore, is to increase the uses of coal.

Research efforts are being concentrated on how to use coal as a substitute for mineral oil and natural gas. The main projects are: production of electricity from coal; production of gas from coal; coal liquefaction; coke production and direct carbonisation; new methods of mining; underground gasification.

Last year, government funds for coal research amounted to DM276 million and will rise to DM380 million in 1978. In the years ahead up to 1981, about DM450 million of government money will be provided. There are also joint projects within the EEC and through the International Energy

Agency, and various agreements with other countries for cooperation on coal research.

West German experience on coal gasification goes back to the Second World War when the bulk of petrol was obtained from coal.

Staff with experience are still available to contribute their expertise. German industrial firms have also built a large number of coal gasification plants all over the world.

One drawback is the high price of German hard coal, so government-sponsored research is being directed at developing economic gasification processes. This year three small coal gasification plants go into operation, one of them a private venture by Shell and Krupp in Hamburg, the other two using government aid. With thoughts of building much larger gasification plant at the beginning of the 1980s, it is highly probable that imported coal will become important. Gasification

plants will be built at German ports to gasify imported coal.

Researchers are also looking at the use of high-temperature nuclear reactors to assist gasification. Reactors could be an economic source of heat for coal gasification. With present methods, more than 40% of the coal used goes in producing the heat necessary for gasification. A reactor could also generate electricity.

Funds for coal research are being channelled through the Federal Research Ministry to a state-controlled management organisation, which selects the projects and grants research funds. Industry's share is small—about 20%—because of the risky nature of coal research, and with very long-term developments the state often bears 100% of the research costs.

Funds for coal research are now greater than those for the promotion of fast breeder reactors. A large number of scientists and engineers are being trained in the new coal research project. The results, though, will not be seen for several years.

Werner Gries

Two reports from the USSR

DURING the past few years, reports of the political misuse of psychiatry to 'cure' symptoms of dissidence, have become frequent. So far, however, it has proved impossible for any western psychiatrist to examine the alleged sufferers—with the notable exception of the 'dissident' who interrupted an international psychiatric symposium in 1974, and was, in fact, genuinely psychologically disturbed (an incident which most western participants regarded as staged).

Recently, however, Dr Gary Low-Beer, a prominent member of the Working Group on the Internment of Dissenters in Mental Hospitals, visited Moscow, *incognito*, as a tourist. While there, he examined nine former psychiatric detainees. In some cases, he says, he found that there was the possibility of some degree of personality difficulty, but in none of them was there any evidence of mental disorder warranting compulsory detention. An example of such a case of mild disorder is that of Petr Starchik, who in the five years preceding his detention had twice voluntarily consulted a psychiatrist for symptoms of depression—a fact which, in Dr Low-Beer's opinion, may have prompted the authorities to choose hospital, rather than prison, as a means of repression.

Others, like Yurii Shikhanovich, the Moscow mathematician, showed no signs of ever having suffered from a mental disorder. Yet, when committed,

Shikhanovich was diagnosed as a "psychopath with the possibility of the onset of sluggish schizophrenia" (the latter 'disease', of course, being unknown to non-Soviet diagnosis).

Dr Low-Beer was unsuccessful, however, in interviewing any current 'patients', although in the case of Evgenii Nikolaev, an associate of the (illicit) First Free Trade Union, now interned in the Kashchenko Hospital near Moscow, Dr Low-Beer was accompanied to the hospital by Mrs Nikolaeva, who was only too willing for him to examine her husband.

The medical director, Dr Murovkin, said that such a visit would be possible only with the permission of the Ministry of Health, an idea which Dr Low-Beer found somewhat ludicrous, because, as he told *Nature*, even if he had pronounced Nikolaev completely sane, this would entail nothing more than a difference of professional opinion.

Dr Low-Beer's interviews with former 'patients' were arranged through the illicit 'working commission on the political misuse of psychiatry', the parallel Soviet organisation to Dr Low-Beer's own. The commission is headed by Aleksandr Podrabinek, a *fel'sher* (paramedic); other members remain anonymous. It is known, however, that several Soviet psychiatrists have now joined the ranks of those who protest against this misuse of their profession.



Shikhanovich: no signs of mental disorder

● Also back from Moscow is John Charap of Queen Mary College, London, who, with three other western scientists, recently visited the Sunday seminar for *refusnik* scientists, now entering its seventh year. Dr Charap assured *Nature* that the seminar is more than a mutual support group—judging from the abstracts of forthcoming papers, some genuine theoretical work of considerable interest is going forward. In particular, the work of Yurii Gel'fand in particle physics (Dr Charap's own field) is well worth the attention of scientists abroad.

He also brought back disturbing news about Leonid M. Borukhin, a metallurgist, formerly at the Research Institute of Nonferrous Metals and

Metal Processing. It appears that the authorities are taking steps to deprive him of his degree of Candidate of Technical Sciences, as a result of his desire to emigrate.

In the past, a few political prisoners and defectors have been similarly deprived. Earlier this year, Arkadii Tsinober of Riga contested the withholding of his doctor's degree on the ostensible grounds that his desire to emigrate made him "amoral and unpatriotic". However, Tsinober had defended his doctor's thesis in 1975, when the new regulations on higher degrees, although not yet in force, were well known to Soviet academic circles. According to these regulations, higher degrees may only be granted to "those who follow the norms of communist morality and those who act according to the principles of Soviet patriotism and proletarian internationalism". The regulations came into force in 1976, and Tsinober might well be regarded as a victim of the changeover.

However, Borukhin received his candidate's degree in 1973, and if he is now deprived of it for his association with the Jewish emigration movement, it would imply that the authorities are prepared to apply the rule retroactively.

Vera Rich

Microwaves may be damaging to health, says US report

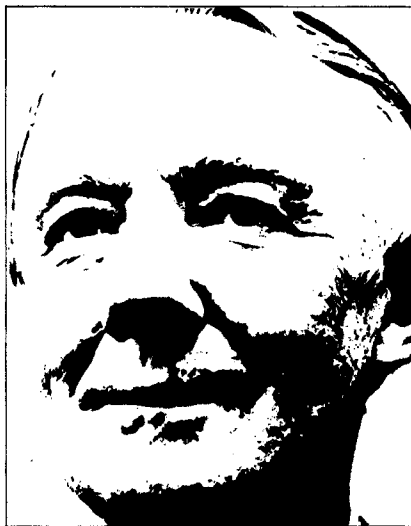
In what has been claimed as the first official recognition of the possible health hazards of microwave radiation, the General Accounting Office—the investigative agency of the US Congress—has concluded that the levels of such radiation to which the public may be exposed could be dangerous.

The GAO, in a report recently made public in Washington, also claimed that the Federal Government has been negligent in monitoring the problem. And Representative Elizabeth Holtzman (Dem.-Brooklyn) has called on President Carter and the Environmental Protection Agency to begin a research programme to determine safe environmental levels of microwave and radio-frequency radiation.

Mrs Holtzman has also asked the president to set up a nationwide monitoring system to determine the extent of actual public exposure, and to continue to maintain a White House office to coordinate the government's policy on microwaves.

Although many scientists consider that present levels of microwave radiation present little public hazard, others claim that those exposed to levels far below the US government's existing standards have experienced symptoms that range from headaches and fatigue to decreased fertility and possibly genetic defects. □

Back to nature



KENNETH MELLABY

CONSERVATIONISTS in Britain and the developed parts of Europe have learned the hard way that nature reserves require sophisticated management. If left alone, they may lose the wildlife for which they were originally scheduled. This is because so few areas are now covered with the natural, 'climax' vegetation, and because we have come to cherish plants and animals encouraged by the farming systems of our ancestors. Thus the attractive flowers of chalk downland only persist so long as the grass is grazed by sheep (or rabbits); otherwise there is an invasion of scrub and, finally, of forest. In some cases these forests resemble those which covered much of Europe three or more thousands of years ago, but in many areas introduced, exotic, trees such as sycamores or conifers from North America take over. The result of leaving an area to nature may be very different from that which existed before man was the dominant factor.

I expected to find things very different in Australia, when making an extensive tour of national parks, nature reserves, forests and farmland earlier this year. Australia, with an area of three million square miles, is sixty times as large as England and Wales, and has only a quarter as large a human population. Although the Aborigines have been there for tens of thousands of years, their numbers were small, and they kept no livestock and did no arable farming. Colonisation from Europe began less than 200 years ago, so agriculture has a very short history. It seemed reason-

able to expect that the greater part of the vast area was still in its natural state, and that the best way to manage reserves would be to leave nature to take its course and to reduce human interference to a minimum.

In some desert and semi-desert areas this policy has generally been effective, but much of Australia has been so substantially modified by man that the results have been disappointing. Although the aborigines may not have practised farming of the conventional sort, they did a great deal of active land management. Large areas of the less-dry country were regularly and skilfully burned, something now described as fire-stick farming. Many indigenous plants flourished after cool, controlled burning, but the development of dense inflammable scrub was prevented, and a sward attractive to grazing kangaroos was produced. Early settlers described much of the landscape of Victoria and New South Wales as resembling "an English country gentleman's park" with large trees set at intervals in lawn-like grass. Little clearing was needed when cattle and sheep were brought in by the early graziers. Some areas have remained in this condition where cattle have cropped the herbage and controlled burning has continued. On the other hand where cattle and fire have been excluded following the declaration of National Parks or nature reserves, an almost impenetrable scrub layer has sometimes developed, with a greatly increased fire hazard. Such conditions, though welcomed by those who prize wilderness, may be far from favourable to many of the endangered species of indigenous wildlife.

Introductions of exotic plants and animals have complicated the situation. Although large kangaroos, which have no predators to control their numbers, may flourish on farmed areas, and may even become serious pests, not all marsupials are so adaptive, and able to compete with aggressive newcomers. Foxes, introduced by settlers nostalgic for the field sports left behind in Britain, and feral cats, escapees from outlying farms, may play havoc among the smaller animals which seem most at risk in the most 'natural' areas. Some attempts to encourage the regeneration of the natural vegetation on farm land absorbed into national parks has resulted in dense thickets of introduced blackberries and an animal population dominated by the exotic rabbit. In fact it appears that many of the conservation problems of Australia have much in common with those of Europe.

correspondence

Palaeoanthropology: more than the sum of its parts

SIR,—I regret that my colleagues were so disturbed by the (perhaps) alarmist title of my article "Is British physical anthropology dying?" (19 January, page 196). I certainly did not intend to under-value their contributions to physical anthropology in its widest sense, but rather to draw attention to the need for institutional resources to be directed to teaching and research in palaeoanthropology. In view of the misunderstanding which my article evoked, I will repeat my propositions in a somewhat different form:

- Palaeoanthropology is today a very different science from the study of fossil man as it was taught 30 years ago. It is now far more than the anatomical study and analysis of fossils and has become a holistic science of early man.
- For this reason it should find its home in anthropology departments where palaeoanthropologists can rub shoulders with archaeologists and social anthropologists, as well as all other kinds of physical anthropologists.
- I believe that palaeoanthropology is now a central part of the science of man and the most fundamental of the anthropological sciences: the present can only be understood as a product of the past. It requires far greater representation in our curricula.

The situation described at University College seems to me almost ideal—except in one respect: that as a result of "historical circumstances" palaeoanthropology is taught by two "temporary lecturers" (who both happen to be US citizens). This exemplifies perfectly the point I was trying to make: we desperately need further institutional support for palaeoanthropology in this country.

Very valuable research is being carried out in both museums and anatomy departments—research appropriate to these establishments and essential to the progress of palaeoanthropology. But I believe that today palaeoanthropology is more than taxonomy, more than anatomy, and it is more than human biology. It is a rapidly developing and increasingly popular discipline. It is the framework for the study of mankind, as it traces the history of human anatomy, behaviour, society, technology and ecology—all part of the complex system of human evolution.

Yours faithfully

B. G. CAMPBELL

Hunstanton, Norfolk, UK

Les écologistes and the French elections

SIR,—We were surprised by the article 'Ecologists lose in French elections'

(30 March, page 393). The author is free to give his own interpretation about the ecological results during the elections, but, in a scientific magazine, he must be careful about fact.

● There has never been an ecological group called 'les Verts' (the Greens). It is the popular name in France for all ecologists.

● The ecologists never obtained 5% of the votes during the municipal elections last year, but a little more than 1% on a national level, because they did not have many candidates.

● This year, they doubled the score with 2.2% of the votes.

● Brice Lalonde never called to vote on the second turn for the left-wing opposition.

What is true is that, each ecological candidate won on average 5% of the votes in his area. There were about 200 ecology candidates in 500 electoral areas. This was less than during the municipal elections.

Yours faithfully,

JEAN-JACQUES PORCHEZ

Les Amis de la Terre de Paris, France

Saccharin—the risks and benefits

SIR,—We were interested in Dr Cohen's comments (9 February, page 492). Cohen quotes our paper (*Lancet* 17 September, 1978, page 578) as providing data on the risk of bladder cancer if the United States population were to ingest one diet soft drink each day throughout their lives. We question his conclusion that this would induce an extra 1,200 bladder cancers per year, primarily because we doubt whether the data available on the carcinogenicity of artificial sweeteners is in an appropriate form to perform risk-benefit analyses, as too many extrapolations are required.

An example of an analysis which might be performed which is at least as defensible as that of Dr Cohen is given in the table. We have assumed: that the risk for males who consumed artificial

sweeteners in tablet or drop form in our study can be extrapolated to the consumption of diet soft drinks; that individuals commence consuming the equivalent of one tablet of saccharin a day from the age of 16 and continue this throughout their lifetime; that there is a twenty year latent period for the effect; and that there is a linear dose-response relationship permitting extrapolation of our data to younger and to older ages.

Applying the rates of bladder cancer for all races and centres in males from the US Third National Cancer Survey and the calculated risk ratios to the population of males in the United States from the 1970 census (third column of the table) we derive an expectation for a total of 67,309 cases with saccharin to 19,626 without. As the rates we use are mean annual incidence rates, the excess cases in males from consumption of saccharin could be 47,000 a year. Thus, any comparison with obesity must be regarded in a different light even if one is prepared to concede the debatable point that diet soft drinks are valuable as a means of combating obesity.

In addition, however, the population is not exposed to artificial sweeteners only through consumption of diet soft drinks. As a result of the publicity that followed our results, we learnt that the food industry was planning the introduction of a new generation of prepared foods for which the sweetening agent was to be saccharin rather than sucrose. The reason for the preference was not a desire to reduce the risks of obesity to the population, but rather that sucrose is more expensive and therefore the potential economic gain would be greater if artificial sweeteners were used. Our concern, therefore, even though we do not claim that our results are definitive (*Lancet*, 10 December, 1977, page 1221) is over the unwitting exposure of large populations to a potentially hazardous substance. The banning of saccharin as a food additive is, therefore, an appropriate course of action.

Yours faithfully,

A. B. MILLER

GEOFFREY R. HOWE

National Cancer Institute of Canada,
Toronto

Incidence of Bladder Cancer and Effect of Regular Consumption of Saccharin
(equivalent to one tablet a day from the age of 15)

Age group	Rate per 100,000	Population (US males)	Number of cases	Cumulative saccharin consumption	Risk ratio	Number of cases (with saccharin)
35-39	3.0	5,412,423	162	8,218	1.5	243
40-44	6.8	5,818,813	396	10,044	1.8	713
45-49	13.4	5,851,334	784	11,871	2.1	1,646
50-54	23.1	5,347,916	1,235	13,697	2.4	2,964
55-59	39.0	4,765,821	1,859	15,524	2.8	5,205
60-64	65.9	4,026,972	2,654	17,350	3.1	8,227
65-69	106.5	3,122,084	3,325	19,177	3.4	11,305
70-74	145.2	2,315,000	3,361	21,003	3.7	12,436
75+	196.4	2,978,624	5,850	23,741	4.2	24,570
Total	—	—	19,626	—	—	67,309

news and views

Stereoscopic visual processing

from J. Pettigrew

THE power of human stereopsis can be demonstrated if you can obtain two copies of your daily newspaper which rolled off the presses at different times. When each copy is presented simultaneously to a different eye and fusion obtained, minute differences produced by wear of the typeface during printing will be seen as alterations in the apparent depth of different letters. Such tiny differences between the retinal images of each eye—retinal disparities—can be detected by the visual system with an accuracy more than ten times finer than the diameter of the smallest retinal photoreceptor. The separation of the eyes in the head determines the angular size of disparities which are useful to an animal for depth judgements. In view of their small size it is not surprising that physiological measurements of the response of single neurones to such small disparities have been attended by considerable technical difficulty and some degree of controversy.

At a recent meeting in London* progress in this area was reviewed by P. O. Bishop (Canberra) in whose Sydney laboratory disparity-selective binocular neurones were first found in cat striate during the early 1960s. Subsequently the laboratories of both Bishop and H. B. Barlow undertook a determination of the range of variation in preferred disparity amongst binocular neurones. Discrepant values for this variation were obtained and a third laboratory even claimed to find no significant variation at all. At the meeting, Bishop undertook to resolve these differences with a consideration of the systematic changes in disparity which occur with changes in position in the visual field. Because of these changes, the exact value obtained for the disparity variation depends very much on

the location and extent of the visual field region represented by the sample of neurones studied. While the variation is large enough to be readily measurable in the periphery and becomes very obvious if data are pooled from widely different eccentricities, extraordinary care is required in binocular stimulus control if the variation is to be demonstrated in the centre of gaze where the noise introduced by residual eye movement, for example, has a magnitude approaching that of the disparity phenomena under study.

This problem is magnified further in the monkey, whose resolution exceeds that of the cat by a factor of five or more. Disparity-selectivity and variation were thought not to be found in monkey striate cortex, on the basis of studies of the paralysed preparation, but new data from behaving animals presented at the meeting by B. Fischer (Freiburg), indicate that monkey and cat striate cortex are actually quite comparable with respect to binocular disparity processing if one takes into account the increased resolution of the monkey. The technical advance made by Fischer, working with G. Poggio (Johns Hopkins University) was the use of a binocular stimulus which moved in real depth and could therefore provide reliable disparity variations down to a few minutes of arc. The problem of the residual eye movements of paralysis was eliminated by the use of an awake monkey trained to fixate a small spot with both eyes while the binocular neurone under study was investigated with a bar stimulus at different depths. Just how stable is eye position in these circumstances remains to be demonstrated directly, but the different, and extraordinarily sharp, depth profiles which can be reproducibly obtained from different neurones argue that the monkey's voluntary control over binocular alignment operates over a narrower range than the

total variation seen in optimal disparity from neurone to neurone. As in the cat, this variation increases with eccentricity. The majority of neurones had preferred disparities clustered around zero disparity, although one would guess, bearing in mind the seconds of arc accuracy of stereopsis, that many of these will prove to have optima significantly different from zero if future techniques can push past the current limit of accuracy at three to four minutes of arc.

Another way around the technical problems inherent in the measurement of small disparities was provided by the work of D. Whitteridge (Oxford) and his collaborators on the sheep. The particular technical advantage of this animal lies in the wide separation between the eyes, which produces retinal image disparities in angular terms more than three times those produced by comparable object disparities in cat or monkey. P. Clarke (Lausanne) described work on the visual cortex of the adult sheep which not only demonstrated disparity-selective neurones with different preferred disparities, but which indicated that, within the secondary visual area (area 18), there may be three separate groups of disparity-selective neurones, each occurring in clusters or even 'columns' and each preferring targets respectively, nearer than, farther than, and centred on, the fixation plane. Hints of such an arrangement have been provided in the past, both from some physiology on cat cortex (C. Blakemore at Cambridge), from psychophysical studies on stereo-blind humans (Richards at Massachusetts Institute of Technology) and from theoretical considerations (Marr at Massachusetts Institute of Technology), but the data from sheep

*The Royal Society held a meeting on Stereoscopic Vision in London on 9 March 1978.

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represent the first clear indications that the central organisation of disparity-detectors may be arranged into three pools, like the tripartite arrangement of colour-sensitive cones.

The sheep, or rather, the lamb, also provided some grist to a mill which was set turning in the last century by the arguments of Hering and Helmholtz about whether stereopsis is innate or acquired. Abnormal visual experience has been shown by numerous experiments, beginning with those of Wiesel and Hubel in 1963, to have enormously powerful effects in a number of mammalian species on cortical binocular connections, the very ones required for disparity-selectivity and therefore for stereopsis. However, controversy rages over the importance of normal visual experience during development, one contentious issue here being the degree of functional sophistication exhibited by visual cortical neurones when these have been examined in young, visually-naïve animals by different groups of experimenters (reviewed by Blake-More). Differences of opinion about young animals may not be too surprising, in view of some of the changing ideas on adult binocular visual processing resulting from the technical advances mentioned above, and so a study of a young lamb's visual cortex is particularly welcome in the light of the relatively clear picture which seems to have emerged for the adult sheep. Clarke and V. S. Ramachandran (University of Cambridge), working with Whitteridge, provided some observations on the newborn lamb's visual cortex which might satisfy both Empiricist and Nativist points of view. Apparently disparity-selective binocular neurones, covering a wide range of different preferred disparities, are present in the second visual area of young lambs which have had no previous visual experience, in support of the Nativist view that some of the basic machinery for stereopsis is innate. On the other hand, one class of binocular neurones, dubbed 'AND' gates because they respond only to simultaneous binocular stimulation, seemed to be absent from the visually-naïve lambs, suggesting that visual experience is important for the development of these more sophisticated processors of binocular information. The underlying connectivity responsible for a binocular 'AND' gate is not clear, but the view that they represent a higher level of binocular information processing and the view that they may require visual experience for their development received support from a rather unexpected quarter at the meeting, from work on the owl's visual system.

That owls have any binocular visual processing at all should come as something of a surprise to some workers,

Methanogenic bacteria

THE question of whether methanogenic bacteria might constitute a third form of life has received considerable coverage recently in the scientific press^{1,2}. The two publications which initiated this discussion^{3,4} present oligonucleotide catalogues of the small-subunit ribosomal RNA from 10 species of methanogens and argue from these and other data that the methanogens represent a primary kingdom distinct from the typical bacteria (eubacteria) and from the eukaryotes. In the comparison of the various 16S and 18S ribosomal RNA sequences, however, one salient point seems to have escaped notice.

I refer to the only portion of the ribosomal RNA whose role in the translation process has been identified. Over the past four years there has accumulated substantial evidence that messenger RNA binding to the *Escherichia coli* ribosome is mediated in part by base pairing between a pyrimidine-rich sequence near the 3' end of 16S rRNA and a purine-rich region preceding the initiation triplet^{5,6}. Examination of the current collection of over 40 different initiator

regions from phage and *E. coli* mRNAs (see ref. 6 for a partial listing) reveals that the rRNA sequence CCUCC is most consistently utilised in this important RNA-RNA interaction. All other species of eubacteria analysed so far seem to retain at least four of these residues in a comparable position near the 3' end of their 16S rRNAs^{7,8}.

Newly determined sequences of 18S rRNAs from six species representing diverse branches of the eukaryotic kingdom also show strong homology to *E. coli* 16S rRNA in the 20 residues at the 3' end⁹⁻¹¹. However, in every case the entire CCUCC sequence is deleted, suggesting that the eukaryotic ribosome has evolved an alternative mechanism for specific recognition of mRNA initiator regions.

Fox *et al.*³ show that 16S rRNAs from all 10 species of methanogens possess the 3' terminus AUCACCUCCU_{OH}. This sequence contains the prototypical and functionally important CCUCC and is conserved despite variations in virtually all other large 16S oligonucleotides. Thus, it is difficult to concur with Woese and Fox⁴ that "there is no reason at present to consider methanogens closer to eubacteria than to the 'cytoplasmic component' of the eukaryote." Rather, a high degree of functional relatedness between the methanogenic and other 16S rRNAs is indicated.

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who have pointed out that, as in other birds, all the information from each eye of the owl crosses to the opposite side of the brain. Birds do not have partial exchange of information at the optic chiasm like that which permits binocular interaction in mammals. At the meeting J. D. Pettigrew (Caltech) described how the owl achieves binocular visual processing with a second optic chiasm, which permits a bilateral projection from the thalamus to the Wulst, a forebrain structure where there are binocular visual neurones almost indistinguishable from those found in mammalian visual cortex. The convergence of function in owl Wulst with that found in cat, monkey, and sheep visual cortex was particularly surprising in view of differences both in the arrangement of the visual path-

way, and in the morphology of the neurones involved, since the owl Wulst, to take one example, lacks the pyramidal neurones with long apical dendrites which characterise mammalian cortex. Not only were all the various classes of neurones with orientation-sensitivity, length-sensitivity, disparity-sensitivity, and so on, represented within the Wulst, these neurones were also very sensitive to early visual experience. Particularly sensitive to experience are the binocular 'AND' gate neurones, which are located in the superficial laminae of the Wulst and therefore probably identifiable with the output neurones seen anatomically at the highest level of processing.

Further study of the properties of higher-order binocular neurones such as the 'AND' gate neurones, as well as

revealing more details about the much-argued role of visual experience in stereopsis, may also help in the resolution of a problem which came up repeatedly in discussion at the meeting and which has eluded all investigators during the past decade's research on disparity-sensitive binocular neurones. The problem concerns the way in which a global synthesis of an unambiguous depth plane is achieved from local detectors of different horizontal and vertical disparities. Although vertical disparities do occur between the retinal images and therefore must be provided for by a range of binocular neurones with different preferred vertical disparities, it is only the horizontal disparities which provide a cue to depth. A final account of stereopsis in physiological terms will therefore require a description of neural processing which is specific for a particular horizontal disparity but generalises over vertical disparity, and which resolves the ambiguities present at the local level by also generalising over a fairly large area of visual space. If single neurones with such global attributes are to be found, one expects them to occur in 'third tier', a complex agglomeration of different visual areas lying in front of the primary and secondary visual cortices. Exploration in this complex area has begun and S. Zeki (University College, London) described the combined anatomical and electrophysiological approach with which he has been able to define four new, distinct visual areas in the macaque monkey. While random-dot stereograms have not yet been used to search in these areas for neurones involved in global stereoscopic processing, A. Cowie (University of Oxford) presented behavioural evidence suggestive of the presence of such neurones in a 'higher' visual cortical region around the anterior end of the superior temporal sulcus. Monkeys with lesions in this area appear to perform normally on 'local stereopsis' tasks, where the stimuli are simple bar stimuli like those used to investigate the properties of neurones in the primary and secondary visual cortices, but fail on 'global stereopsis' tasks involving random-dot stereograms where a large number of local comparisons between the two retinal images have to be made, ambiguities resolved and a subset of identical horizontal disparities identified at different lateral locations.

Investigation of 'global stereoscopic' processing at the single neurone level can be expected to move ahead as the sophisticated techniques for presenting dynamic random-dot stereograms become more available to all. Before the days of cheap, large-scale integration of electronic components, the use of computers in animal experiments was

cumbersome and often limited to *post hoc* data analysis. Now many more experimenters can afford powerful systems which enable them to be as sophisticated about stimulus presen-

tation as they were in the past about data analysis. The next decade of research on the neural mechanisms of stereopsis should therefore be as fruitful and interesting as the last. □

Does dietary fat influence plasma lipoprotein structure?

from Anne Soutar

THE arguments in both the scientific and the medical press about the role of dietary fat in the cause or prevention of coronary heart disease show no signs of abating. It is indisputable that in most individuals a marked reduction in the plasma cholesterol concentration is brought about when what has come to be accepted as a 'normal' diet is exchanged for one in which the quantity of saturated fat is reduced and partly replaced by polyunsaturated fat. Moreover, few would argue with the many observations that someone with a high concentration of cholesterol in their plasma is more likely to succumb to premature coronary heart disease than those with a low plasma cholesterol level. Nevertheless, it has yet to be shown unequivocally that it is possible to reverse, or even to retard, the process of cholesterol deposition in the human arterial wall as a result of reducing plasma cholesterol concentration by diet. Thus a chasm still remains between those who believe that consuming a diet rich in polyunsaturated fats increases their chance of avoiding premature coronary heart disease, especially when other risk factors such as smoking or high blood pressure are also present, and those who believe that persuading others to a lifelong abstinence from such foods as butter, cream and cheese is wholly unjustified. In view of the limitations of the experimental evidence, taking up a firm stance on either side requires a certain amount of faith. Certainly our knowledge of the mechanisms by which dietary fat composition affects the plasma cholesterol concentration is far from complete.

The lipids in human plasma are transported in three main classes of lipoprotein particle, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL), each defined in terms of the density of the solution in which they float rather than sediment under an ultracentrifugal force. However, most of the plasma cholesterol, of which 75% is esterified with long chain fatty acids, is carried in LDL and it is a high concentration of this particular lipoprotein in the plasma which is as-

sociated with the incidence of premature coronary heart disease. Thus investigations of the relationship between dietary fat and plasma cholesterol concentrations have inevitably focused on the metabolism of LDL. (However, HDL, which also contains some cholesterol, is currently receiving much attention as a possible protective agent against atherosclerosis).

A diet containing an increased ratio of polyunsaturated to saturated fats undoubtedly results in an increase in the percentage of unsaturated fatty acyl chains in the various lipids in the plasma lipoproteins, and it is well known that the unsaturation of their fatty acyl chains is an important determinant of the fluidity of lipids. Since many membrane processes or enzymic reactions with lipid substrates are known to be profoundly influenced by the fluidity of the lipids involved, one plausible hypothesis for the cholesterol-lowering effect of a polyunsaturated fat diet is that the structure of LDL may be influenced by the fluidity of its constituents lipids in such a way as to influence the rate at which the particles can be catabolised. Alternatively, or additionally, the fluidity of the LDL lipids may affect the rate at which the particle or its constituent lipids are deposited in or can be removed from developing atherosclerotic plaques. Recent reports from the laboratory of D. Small and his group at the Boston University School of Medicine, who have been investigating aspects of the structure of human plasma LDL, provide some experimental support for such a view.

The structural arrangement of LDL particles, in itself a subject of some controversy, is now believed to be similar to that of the other human plasma lipoproteins, in that the particle consists of a spherical core of apolar lipids, in the case of LDL mainly cholesterol esters with some triglycerides, surrounded by a shell one molecule thick of phospholipids. The apoproteins are located in this amphipathic surface layer, together with much of the small amount of free cholesterol in the particle. Small and his colleagues have shown particular interest in the arrangement of the lipids in the core of the particle, having demonstrated that a reversible temperature-dependent

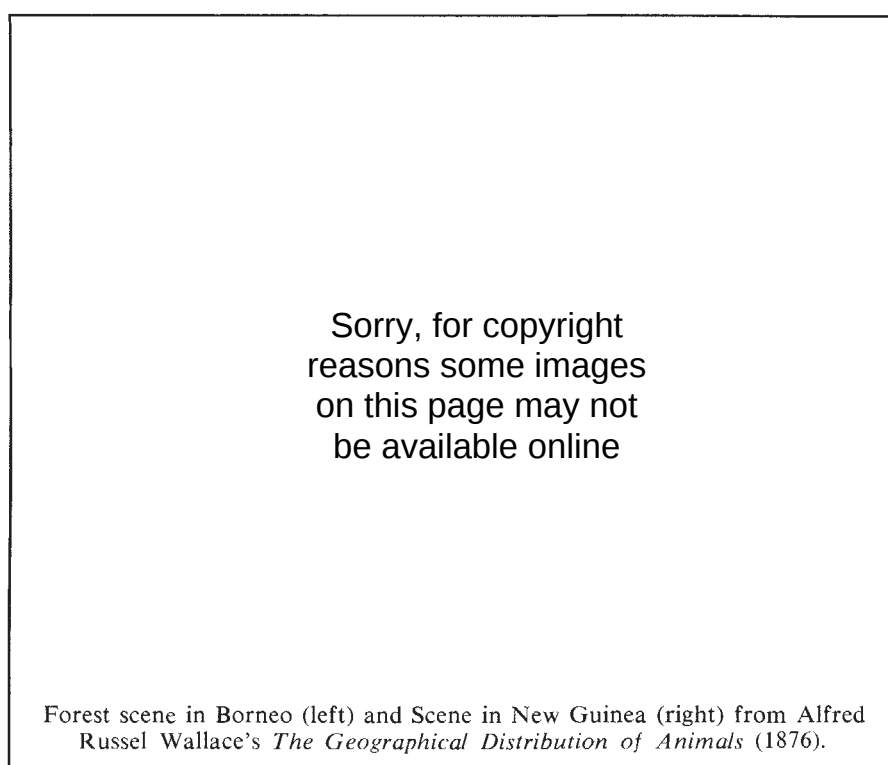
Anne Soutar is a member of the Medical Research Council Lipid Metabolism Unit at the Hammersmith Hospital.

phase transition can be detected in LDL in the region of 20–40 °C by means of differential scanning calorimetry. Since this transition occurs at a temperature close to that at which the cholesteryl esters isolated from LDL change from a liquid crystalline phase to the liquid phase, they deduced that below the transition temperature of LDL, the cholesteryl esters in the core of the particle are able to form a less-fluid liquid crystalline phase (Deckelbaum *et al. J. biol. Chem.* **252**, 744; 1977). Small-angle X-ray diffraction patterns of LDL at different temperatures confirmed these observations, and more detailed analysis of diffraction data by P. Laggner and his colleagues at Graz (Müller *et al. Eur. J. Biochem.* **82**, 73; 1978) suggested that the cholesteryl esters in the core of LDL form two concentric rings when in the more ordered phase.

The temperature at which the peak of the transition from a fluid to the less fluid non-random arrangement of the core lipids occurred for an individual sample of human LDL was less dependent on the nature of the fatty acyl chains of the cholesteryl esters than expected, partly because there was little variation in the unsaturation of the fatty acyl chains between the samples, but correlated closely with the amount of triglyceride in the particle. Thus, LDL samples with the lowest content of triglyceride tended to have the highest transition temperature.

Although the transition temperature of pure cholesteryl esters isolated from the LDL is approximately 42 °C, the effect of the triglyceride in the core is such that in the 21 human LDL samples studied, the transition temperature was always below 37 °C, so that the lipids in the core of the LDL particles would be fluid at normal body temperature. Of particular interest, however, was an LDL sample from a subject with homozygous familial hypercholesterolaemia, in which an abnormally low triglyceride concentration in the core resulted in a transition temperature of 36 °C, close to body temperature. Familial hypercholesterolaemia is characterised by a grossly elevated level of plasma LDL which results from a reduced ability of the tissues to catabolise LDL in the normal way, and invariably is associated with severe premature atherosclerosis. The low level of triglyceride in this sample of LDL was probably the result of the long time spent by the particles in the circulation and thus the low fluidity of the core lipids is more likely to be the effect rather than contributing to the cause of the low rate of catabolism of LDL.

However, whether or not the 'atherogenicity' of the LDL particles is greater as a result of the less fluid



nature of the core lipids remains to be seen, and it would be of interest to determine whether or not there is any correlation between the triglyceride content, and therefore the fluidity of the lipids, of LDL and, for example, the plasma concentration of LDL or its half-life in the circulation in normal subjects. Similarly it is important to determine the extent to which these parameters are affected by diet in normal subjects.

It is in the context of the 'atherogenicity' of LDL that the results of Small and his colleagues, obtained in conjunction with R. Mahley of the National Heart, Lung and Blood Institute, Bethesda, are of interest. Mahley has been investigating the effects of feeding diets rich in cholesterol and saturated fatty acids on the plasma lipoproteins of experimental animals, including miniature swine, whose plasma lipoproteins are more comparable to those in human plasma than are those of many other species, and which readily develop raised plasma LDL levels and severe atherosclerosis when fed such an 'atherogenic' diet. Small and his colleagues found that the LDL from pigs with diet-induced atherosclerosis, as well as the HDL_C, an abnormal lipoprotein which appears in the plasma of animals fed atherogenic diets, have similar structural characteristics to human LDL, with what may be an important distinction, that the transition temperature of the cholesteryl esters in the core of the particles was at or above the body temperature of the pig. In the case of the pig HDL_C and LDL, the high transition temperature could be ascribed

both to the low ratio of triglyceride to cholesteryl ester in the lipoproteins, and to the increased saturation of the fatty acyl chains of the cholesteryl esters.

Thus it is clear that in the plasma of an animal with severe atherosclerosis induced by diet alone, the plasma lipids are transported in a less fluid form than those in normal human plasma (Tall *et al. J. biol. Chem.* **252**, 7288; 1977). Tempting though it may be to infer from this interesting data that there is indeed a correlation between lipid fluidity and the 'atherogenicity' of LDL, it is not yet possible to conclude that this is the case for the normal human subject, or indeed that a change in fluidity is brought about directly by changes in the saturation of the fats in the diet. □

Pre-equilibrium theory

from P. E. Hodgson

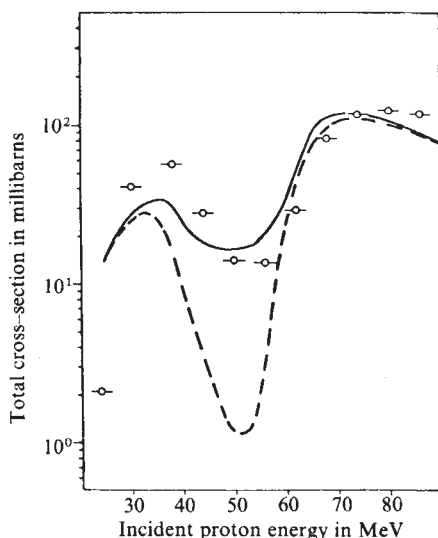
WHEN an energetic proton interacts with a nucleus many nucleons and groups of nucleons are emitted before the residual nucleus reaches its ground state. Measurements of the energy and angular distributions of these particles show that the more energetic ones are predominantly emitted in the forward direction while the less energetic are isotropically emitted and have a Maxwellian energy distribution. It has thus become customary to divide the interaction process into two stages, a direct stage when the incident particle collides with a nucleon or group

of nucleons in the nucleus and immediately knocks them out, and an evaporation stage when the excited nucleus left behind after the direct interactions gradually evaporates particles until its energy is exhausted. The direct stage can be treated theoretically using the nuclear density distributions and effective nucleon-nucleon cross sections, and the evaporation stage by the statistical theory of nuclear disintegration.

On the whole the predictions of these theories agree quite well with the experimental data, but more detailed comparisons show that the distinction between direct and evaporation stages is too sharp; some particles are emitted after the direct process and yet before the establishment of the statistical equilibrium required for the evaporation process. A more microscopic consideration of the interaction shows that the first direct interactions produce two-particle one hole states (2p1h), the next generation of interactions produces 3p2h states and so on until all the nucleons share in the excitation and statistical equilibrium is established. At each stage it is possible for particles to be emitted, and those that come from the decay of the 3p2h states and those subsequent to them before equilibrium is established belong neither to the direct nor to the evaporation stage.

The intermediate stage is called the pre-equilibrium stage, and many attempts have been made to develop the theory of particle emission during this stage so that it can account for the particles observed. Recent work by Gadioli and Gadioli-Erba of Milan and Hogan of Montreal (*Phys. Rev. C* **16**, 1404; 1977) has proved very successful in this respect, and has also provided further evidence for the existence of α -particle clusters in the nucleus.

They assumed that the first interaction of the incident proton gives rise to a 2p1h state, and also allowed for the possibility that it interacts with an α -particle to give 1p-1 α -1 α h states. These states can decay either by particle emission or by the excitation of more complicated configurations. A Monte Carlo calculation governed by a sequence of random numbers gave the probabilities of various possible outcomes at each stage of the process. The following stages of the excitation were included in the calculation and each had the possibility of pre-equilibrium emission, as far as the fourth stage. The cross sections for the interactions and the level densities of the compound nuclei were represented by analytical formulae with parameters adjusted to give the optimum agreement with the known experimental



Total cross section for the $^{90}\text{Zr}(p, 2p3n)$ reaction as a function of incident proton energy. The full and dashed lines are the result of pre-equilibrium model calculations with and without the possibility of pre-formed α -particle clusters in the target nucleus.

data. At each stage in the calculation a random number determines what happens, taking account of the probability distribution of the various possible outcomes. The calculation was repeated a large number of times to give the required cross sections for comparison with the measured values.

Calculations were made for 10 to 100 MeV protons incident upon nuclei in the mass region around $A=90$, and the cross sections of 28 different nuclear reactions were obtained. Typical reactions were $^{88}\text{Sr}(p, 3n)$, $^{88}\text{Sr}(p, 2p4n)$, $^{89}\text{Y}(p, 5n)$, $^{90}\text{Zr}(p, 2p5n)$ and so on for various numbers of emitted nucleons. These numbers refer to the total numbers of nucleons emitted, whether or not they are emitted individually or in composite particles such as α -particles. It is necessary to group the reaction in this way because the experimental technique used in these studies identifies the residual nuclei radiochemically and does not distinguish between the different ways the nucleons can be emitted.

The comparison between theory and experiment for all these reactions shows excellent overall agreement for the total reaction cross sections as a function of energy. A typical cross section first rises rapidly as soon as the incident energy exceeds the threshold, and then passes through a broad maximum before falling again as other reactions with higher thresholds become possible and start absorbing more and more of the incident flux. In some of the reactions a more com-

plicated behaviour is observed, and this can be understood by taking into account the possibility of α -particle emission. Thus the cross section for the $^{90}\text{Zr}(p, 2p3n)$ reaction shown in the figure has two peaks. The first is due to the $(p, \alpha n)$ reaction and the second to the $(p, 2p3n)$ reaction with the nucleons emitted individually.

The only adjustable parameter in this calculation is the probability for the pre-formation of α -particles, which determines whether these particles are emitted in the pre-equilibrium stage. Comparison with the data indicates a value of about 0.15 for this probability. Also shown in the figure is the result of a calculation with this probability set to zero. This shows the necessity of including the possibility of pre-formed α -particles to account for the value of the cross section in the energy range between the two peaks.

The theory of pre-equilibrium emission has thus reached the stage of reliable quantitative calculations, and has added to our knowledge of α -clustering in nuclei. It is now very desirable to check the theory in more detail by measuring the angular distributions of the individual particles and comparing these with the predictions of the theory. $\square$

Photon fridge

from a Correspondent

PLACE an atom in a high density electromagnetic field and it will experience forces arising from (1) scattering, due to resonance absorption and subsequent spontaneous emission of the light and (2) pondermotive effects due to interaction of the induced atomic dipole moment with the electromagnetic field gradient. Utilising these two forces in a suitable electromagnetic field geometry a 'trap' can be constructed which will cool and retain indefinitely any neutral atomic or molecular species using only radiation pressure forces.

The deflection of atomic beams by resonance laser radiation has been proposed and observed (Ashkin *Phys. Rev. Lett.* **25**, 1321; 1970) in atomic physics scattering experiments. The absorption of resonance radiation and the spontaneous isotropic reradiation by an atom results in a force on the atom in the direction of the incident light. The atom therefore absorbs momentum from the radiation field and heats up. If the frequency is, however, confined to values below that of the Doppler broadened absorption line then the atoms can be cooled. This happens since only those atoms which are mov-

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ing towards the incident laser beam direction find the light Doppler shifted up in frequency and undergo scattering, with a cross section approaching the resonance absorption value. This results in a momentum transfer in the direction opposite to the atomic velocity consequently reducing the atomic kinetic temperature. The atoms moving away from the incident laser beam direction seem out of resonances with the atomic transition and although they will gain momentum from the electromagnetic field the cross section is very small. Hence providing the laser frequency is confined to the lower half of the Doppler linewidth the atoms can only lose momentum by way of the scattering process and never gain it. Hence with the reduction of the atomic velocity the Doppler linewidth can be reduced, in principle, to the natural linewidth of the atomic transition.

The pondermotive force arises from the polarisability of the atom in the high intensity electric field gradient in the laser beam. This force, which is dependent on the square of the electric field, will either move atoms into or out from regions of higher field intensity depending on whether the frequency of the radiation is less than or greater than the resonant frequency of the transition (Gordon *Phys. Rev.* **8**, 14; 1973). So in a single laser beam confined to subresonance frequencies the atoms will be pulled towards the laser beam axis and trapped, analogous to the trapping of positive ions by space charge in an electron beam.

By combining these two effects atoms can be radiatively cooled and trapped in a high density electromagnetic field. The efficiency of the cooling trap will depend on its geometry and Ashkin (*Phys. Rev. Lett.* **40**, 729; 1978) has proposed a configuration which produces a continuous wave trap capable of cooling, and retaining, as many as 10^7 atoms to 10^{-6} K (equivalent to a single optical photon momentum). This proposal based in a new treatment of the saturation of these forces provides a trapping energy 100 times greater than that previously envisaged.

Two converging laser beams, both in the TEM₀₀ mode, are directed along the same axis in opposite directions. The 200 mW focused beams waist and subsequently diverge, forming the trapping volume in the light beam overlap region between the waist points. The centre of the trap forms a point of stable equilibrium. If a collimated beam of sodium atoms is injected into the trap along the axis of one laser, which is tuned to a frequency, f_n , 50 times the natural width below the D line resonance frequency f_0 , they will execute Doppler damped oscillators along the axis until they are cooled

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FUSULINIDS (x 10) from the Furner Valley Limestone (from Morris, Douglas & Kopf, *US Geological Survey Professional Paper*, 1025). These complex marine foraminifera, some reaching up to 1 cm in length, flourished in the Carboniferous and Permian. The different genera are distinguished on the basis of the internal structure.

and held by the pondermotive forces in the trap. The interference of the two laser beams produces a system of standing wave fringes within the trap region and these axis field gradients will attract (since $f < f_0$) atoms to the fringe peaks and hold them there. Once trapped on a fringe the atoms continue to be cooled by Doppler damping down to a single photon momentum $\sim 10^{-6}$ K.

If the waist diameters of the focused beams are 12 μm and the separation of the waist positions is 2 cm then the trap is sufficiently strong to cool sodium atoms from 300 K ($6 \times 10^4 \text{ cm s}^{-1}$) to 6×10^{-7} K (3 cm s^{-1}) at a trapping rate of $10^6 \text{ atoms s}^{-1}$. The overall radial pondermotive forces are sufficiently strong to trap atoms with transverse velocities up to 200 cm s^{-1} . In order to

remove all transverse kinetic energy by radiative cooling two additional pairs of opposing laser beams would be required to form a three-dimensional trap. However, once atoms are trapped collisions between atoms should thermalise all velocity components to 3 cm s^{-1} .

These cooled atoms can be used to investigate atomic structure essentially free of Doppler effects or the interactions of very slowly moving atomic and molecular species. If atoms could be velocity selected before injection into the trap then the trap could also be used to study the radiative scattering effects themselves. In a more applied application the radiation pressure could be used to collimate atomic beams by selectively cooling the transverse velocity component. □

review article

Scanning electron microscopy and the surface morphology of human lymphocytes

Stuart Roath*, Diane Newell*, Aaron Polliack†, Elaine Alexander‡ & Peck-Sun Lin§

The surface of the human lymphocyte as seen by the scanning electron microscope shows variations which may reflect the functional state or environment of the cell. Preparative techniques and future developments in this area are reviewed.

RECENTLY there have been several attempts to use the scanning electron microscope (SEM) to describe the surface topography of human circulating and tissue cells—including lymphoid cells derived from normal blood and tissues, and abnormal, usually neoplastic, states. During this same period there have been several simultaneous developments not only in the techniques associated with the use of the SEM but also in concepts of lymphocyte function. First, commercially available SEMs now offer a resolution better than 50 Å compared with the 100–200 Å obtainable on the previous generation of machines. Second, improvements in the preparation of 'soft' biological material for the SEM, especially in the use of critical-point drying techniques and sputter-coating, and improved methods of separating and concentrating lymphocytes from whole blood have come into general use¹. Third, the development of techniques to classify and 'purify' the two main lymphocyte populations, the T and B cells, have all occurred relatively recently so that it has been possible to study 'pure' preparations of lymphocyte subpopulations relatively free from contamination with other leukocytes². The understanding of lymphocyte movement, circulation and surface behaviour has also improved.

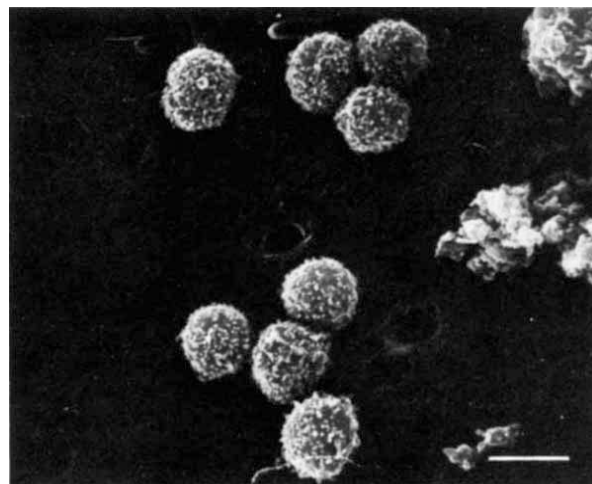
Although, because of methodological variation, it has been difficult to compare data from different laboratories, there have been clear areas of gain overall—the photographs of lymphoid cells shown in atlases of fine structure of only a few years ago^{3,4} have been superseded by technically much improved views^{5,6} with greater and clearer detail. Even so publication of photographs demonstrating either techniques generally regarded as obsolete⁷ or errors in morphological attribution⁸ are still occasionally seen.

The absence of a generally accepted technique for lymphocyte SEM preparation has led to problems in comparing work from different sources^{9–13}. This probably accounts for conflicting reports on differences in the surface of T and B lymphocytes. No obvious morphological differences had been established by light microscopy or transmission electron microscopy (TEM)¹⁴, and it now seems that any differences seen in SEM pictures were probably due either to the functional state of the cell, or to its place in the lymphocyte differentiation pathway or its response to (an unsuitable) environment during processing. The

contradictory reports concerning lymphocyte surface features^{12,13} suggested that careful evaluation of the preparative techniques used was needed before a 'final' assessment of surface morphology could be made and that results obtained from animal work may differ from that in man^{15,16}. However, other techniques, such as differential interference microscopy or immunoelectron microscopy may still show morphological differences between T and B lymphocytes. Polliack¹⁷ also records thymocytes as being different (smoother) from peripheral blood lymphocytes regardless of preparative methodology.

Before discussing any specific variations in lymphocyte form it is appropriate to describe the currently accepted normal range of lymphocyte surface morphology. It is generally accepted that most circulating 'normal mature lymphocytes' are spherical with a mean diameter of 4.9 µm and a range of about 3.5–7.5 µm. This is smaller than that usually accepted for circulating peripheral blood lymphocytes and can be accounted for by shrinkage during glutaraldehyde fixation and critical-point drying. The size shows a random distribution within these levels, and the lymphocyte surface is covered with microvilli, also randomly distributed. These microvilli vary in length (0.2–1.2 µm) and thickness (0.2–0.5 µm) and are mostly in a constant numerical relationship with the cell surface, although an occasional smooth or scantily villated cell may be seen¹⁸ (Figs 1–3). In a recent report on normal lymphocytes¹⁹, gradation of microvillous density and size was considered sufficient to

Fig. 1 Normal peripheral blood lymphocytes. Scale bar, 5 µm.



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allow lymphocytes to be classified into three types; however, these did not seem to correspond with known lymphocyte subpopulations.

It should be emphasised that the possession of some microvilli is a common feature of many blood leukocytes^{19,21} and freed tissue cells²². Granulocytes and monocytes however, characteristically show many frond-like surface projections which are not thought to be normally present in lymphocytes, except possibly in a very small (about 1%) subpopulation.

Causes of variation

The factors known to cause variation in the lymphocyte surface are as follows. First, there are several known artefacts of processing. Generally speaking, cell insult or injury initially leads to surface smoothness, followed by 'cracking' or pitting. Separate processes in cell handling for the SEM can be individually considered for their effect on the lymphocyte surface and cell viability and recovery should be checked at each stage. It seems that the osmolarity and ionic composition of fixative buffer solutions are important. Inadequate fixative concentrations may allow surface changes to occur during subsequent manipulation. Even 2% glutaraldehyde allows morphological changes for about 2 min. The osmolarity of the fixative vehicle is critical^{23,24} for correct surface preservation. Recently it has also been demonstrated that aldehyde fixations can cause surface blebbing²⁵.

Observations made on cells fixed while still cold or shortly after processing at low temperatures, may show a smooth state, due to retraction of microvilli. Although this process may be reversible before fixation, the lymphocytes could be fixed in any degree of villous contraction²⁶. Variations in temperature during experiments immediately before cell fixation may well account for the reported differing surface morphology of rosetted T cells²⁷⁻³⁰ where rosetting took place at 4° or 20 °C. Finally, the use of air-drying techniques³¹ leads to cell spreading, loss of spherical shape and collapse of microvilli⁹. Many reports on cells (and tissues) so processed are still available in current literature.

Contact seems to cause redistribution of villous material in whatever cell is observed³². Cell-cell contact, as in lymphocyte rosette formation of any type, seems to induce lengthening and redistribution of microvilli and perhaps withdrawal of villous cytoplasm³³. Contact with 'neutral' surfaces such as glass or nylon fibres³⁴ produces a similar result. Contact with biologically active materials such as endothelium³⁵ may induce villous formation. In the appropriate circumstances, immobilised antigen complexes³⁶ cause dramatic surface changes including cell shape and the redistribution of microvilli (Fig. 4).

It seems likely that exposure to heavy metals produces villous retraction but this is probably a toxic effect of the metal itself rather than a contact effect since it seems to lead to other surface changes such as the pits and cracks indicative of cell death¹³. This may account for some of the morphological changes reported when metallic filters are used in processing cells which are not—or insufficiently—prefixed^{15,12}. E. A. and Wetzel¹¹ have described serial charges induced by time-dependent contact with silver membranes. As might be expected these contact-induced changes vary according to the proximity of the cells to the membrane, thereby artefactually suggesting a mixed population. Observations such as these stress the importance of proper fixation in suspension, temperature control and processing by techniques which do not result in cell distortion or loss.

Earlier reports of the actions of mitogens, involving the examination of air dried cells³⁷, have not subsequently been substantiated. A. P.³⁸ using phytohaemagglutinin (PHA), pokeweed (PWM), concanavalin A (con A) and phorbol myristate acetate (PMA), and D. N. and S. R.³⁹ using

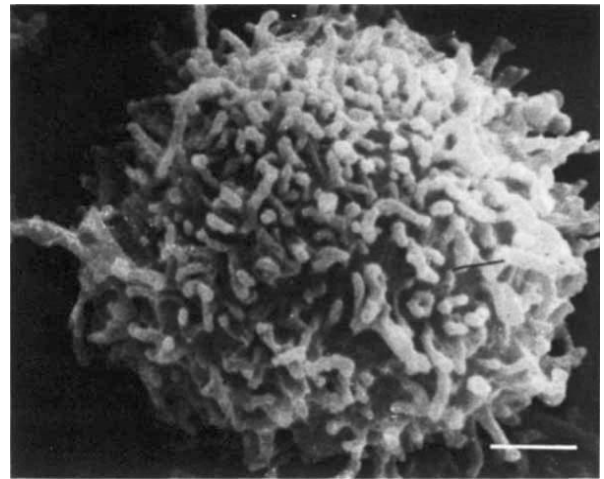


Fig. 2 Normal peripheral blood lymphocytes with numerous long microvilli. Scale bar, 1 μ m.

PHA, PWM and anti- β_2 -microglobulin have shown that sequential changes occur during the culture period (usually 72 h): an increase in cell size is observed on many of the cells, uropod formation is noted, microvilli which are well developed in the early period of culture decrease, and the cells tend to clump together. There is a certain variability in the appearance on the individual cells; how much of this is due to specific responses to the various mitogens or the variable response by individual cells cannot be ascertained. Adequate data are now available to confirm that B and T cells can be specifically stimulated by appropriate mitogens, accounting perhaps for these changes. Most of the mitogens that have been used so far are probably insufficiently selective although anti- β_2 -microglobulin may prove to be different. It is a matter of conjecture whether lymphocytes from special tissues or from subjects with lymphoproliferative disorders may have a surface morphology which is reflected in these *in vitro* changes. Such a relationship might be in keeping with some current ideas on the classification of lymphocyte malignancies⁴⁰.

It has been demonstrated, that muscle poisons such as vincristine or its derivatives and colchicine, are active at the cell surface and inhibit the formation of microvilli, presumably by reacting on the known cytoskeletal membrane-associated elements involved in cell surface motility^{41,42}. Fagraeus and her colleagues^{43,44}, using indirect immunofluorescence have shown the presence of contractile tissue (microtubules) both in cultured haematopoietic cell lines and in circulating human blood lymphocytes and lymphoid cell lines. Cytochalasin B has also been shown to modulate agglutinability by alteration of the surface topography of non-lymphoid cells⁴⁵ and is equally effective on the lymphocyte surface⁴¹. Loor⁴¹ has also suggested a relationship between the ring-patch-cap transition phenomenon and cell surface villous control. However, such drugs appear to have little effect on lymphocyte microvilli that are already formed⁴⁶. The physiological processes equivalent to *in vitro* events such as these, and others probably not identified, may be reflected in the range of morphology seen in the circulating lymphocyte. This variation may therefore be due to the state of activity of any lymphocyte studied, for example, whether it has recently contacted other cells or antigen/antibody complexes, or is undergoing differentiation. Tissue (nodal, splenic) lymphocytes are more difficult to assess as, presumably because of their intimate cell-cell contact, their surfaces tend not to show regular features or to be consistently villous. The process of obtaining free cells from tissues is usually somewhat traumatic and may account for the rather irregular surface appearances described. Whether allowing these cells to 'settle' by

incubation *in vitro* for long periods after their disassociation from their tissues would reflect a 'true' surface appearance has yet to be investigated. However, thymocytes have consistently been shown to be smoother than peripheral blood lymphocytes regardless of preparative methodology³⁸.

Surface morphology in abnormal lymphoid states

There are as yet relatively few publications on sizeable numbers of individuals who have had abnormal circulating lymphocyte states and/or disorders of lymphoid tissue. In chronic lymphatic leukaemia it is suggested that there is a variable but overall tendency towards the lessening of numbers of lymphocyte microvilli^{16,47}, although this is denied elsewhere^{12,50}, while in acute lymphocytic and other leukaemias idiosyncratic cell forms are described⁴⁸. The cells described in hairy-cell leukaemias⁴⁷ seem to be morphologically diagnostic with their 'hybrid' monocytoid/lymphoid appearance⁴⁹, and the so-called characteristic cells seen circulating in the Sézary syndrome actually show quite gross abnormalities of size, shape and distribution of microvilli which vary from patient to patient. Polarisation of villi and uropod formation has also been reported in this disorder^{18,51,52}.

Tissue lymphoid cells from patients with lymphomas have been variously described but insufficient confirmed evidence of specific morphological features in human tissue lymphocytes in patients with lymphomas is available⁵³. The occasional unusual circulating cell in these disorders may not be diagnostic of specific underlying disease such as lymphoma since these can be seen, albeit rarely, in normal peripheral blood and great care must be taken in assessing morphological data in such disorders because of the preparation of artefacts already mentioned.

Lymphocyte surface marking by SEM

The agents used for lymphocyte surface marking by light microscopy, for example, fluorescent-tagged immunoglobulins (or immune complexes), are unsuitable for SEM identification and those used by the TEM, for example, peroxidase and ferritin, are not within the useful range of resolution of the SEM^{54,55}. 'Sandwich' labelling techniques involving macromolecules such as latex spheres or large viruses^{56,57} such as the TMV or bushy stunt virus, have been used with some success⁵⁸⁻⁶⁰. Although there seems to be considerable potential here for investigating in detail the sequential events in information reception and handling by lymphocytes and possible concurrent changes in surface, the techniques described are yet unstandardised and complex and such 'markers' may themselves alter the cell surface.

Fig. 3 Normal peripheral blood lymphocytes with many short, stub-like microvilli. Scale bar, 1 μ m.

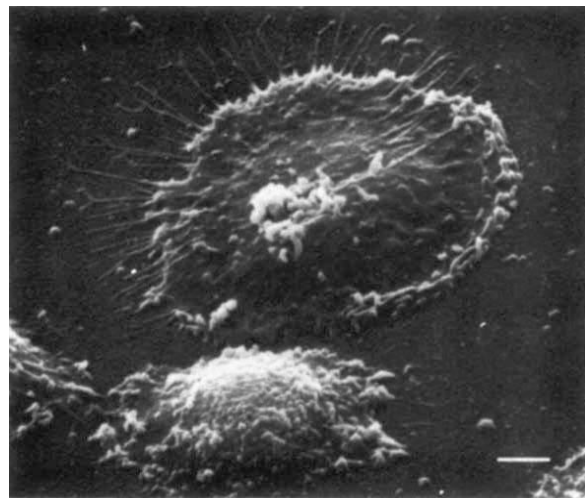
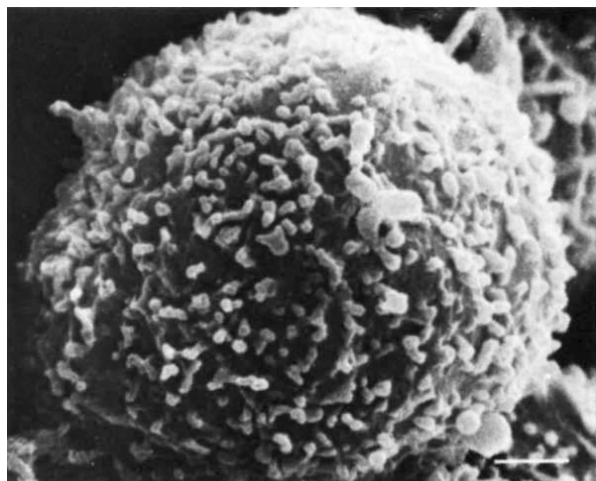


Fig. 4 A flattened and elongated adhered normal Fc-bearing lymphocyte adherent to immobilised antigen-antibody complexes. Scale bar, 2 μ m.

The future of the SEM in lymphocyte biology

The normal fine-surface anatomy of the lymphocyte now seems to be satisfactorily defined by the SEM, and its sensitivity to change in its environment appreciated. The surface alterations so far described seem to result from environmental, functional or malignant changes rather than taxonomic differences. Consequently the following potential uses of the SEM can be envisaged. First, the identification of abnormal lymphoid (or lymphoid/monocytoid/plasmacytoid variant) populations in circulating blood, and the study of tissue lymphocytes in abnormal lymphoid states (lymphomas and reactive states) can be assessed. Second, the morphological changes associated with the function of the lymphocyte surface in, for example, antigen-antibody complex, complement and mitogen reception, can be investigated further, thereby increasing understanding of the immunological processes involved.

The resolving power of the scanning electron microscope seems to be subject to continuous improvement so it would seem that changes in the lymphocyte surface could be recorded in much greater detail in the near future. However, improved coating techniques may be necessary before greater resolution is achieved in biological material or the introduction of methods for looking at frozen tissue using the cryostage which may also eliminate potential fixation artefacts. In addition, use of analytical attachments to the SEM such as X-ray analysis may make it possible to identify chemical changes on the lymphocyte surface structure associated with physiological events or abnormal states. Such advances may also allow the use of 'small' markers such as ferritin that are at present only suitable for transmission electron microscopy⁵⁵.

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articles

Pole movement and sea levels

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If the centrifugal force generated by a surface load caused the Pole to shift, sea levels would fluctuate differentially around the world. Application of the 'hydrodynamic' formula to seemingly incongruous shoreline samples dated 14,700 to 28,000 BP suggests rhythmic polar oscillations on a 5,600 yr cycle, synchronised with two glacial episodes.

THE concept that if the Earth developed surface imbalance, shifting of the Pole would result has been discussed by Gold and others¹⁻⁶. However, empirical proof or disproof is wanting. The present analysis seeks to ascertain whether the surface load introduced by the glaciers of the last Ice Age caused detectable changes in the position of the geographic Pole. Putting the question more specifically, can fossil shoreline organisms that are found above or below the present water line provide a gauge to polar movement?

'Pole slippage' means that as slippage occurs the geographical point where the meridians converge spirals away from the axis, circuiting it with each daily rotation of the Earth. We are not concerned here with changes in the posture of the axis. Such changes do not alter sea levels.

The oceans of the world maintain the form of an ellipsoid, with the longer radius at right angles to the axis of rotation. The hydrodynamic hypothesis holds that if the Earth slips its Pole, shorelines will shift, rising or falling by different amounts in different quarters of the globe. Coasts moving towards the Equator will slip under the equatorial bulge of the oceans, and *vice versa*. Opposite changes will occur north and south of the Equator. However, the changes are opposite only in the ad-

jacent 'quartersphere', that is, up to 90° on either side of the meridian of polar movement. In diametrically opposite quadrants, similar changes occur. But when the sea level rises in North America, it falls on the other side of the Pole in Siberia.

The potential consequence of polar slippage is substantial. Theoretically (with a rigid crust), a slippage of only 1° would raise or lower sea levels relative to land as much as 373 m. Sea-level changes will be most pronounced along the meridian of polar movement and in latitude 45°.

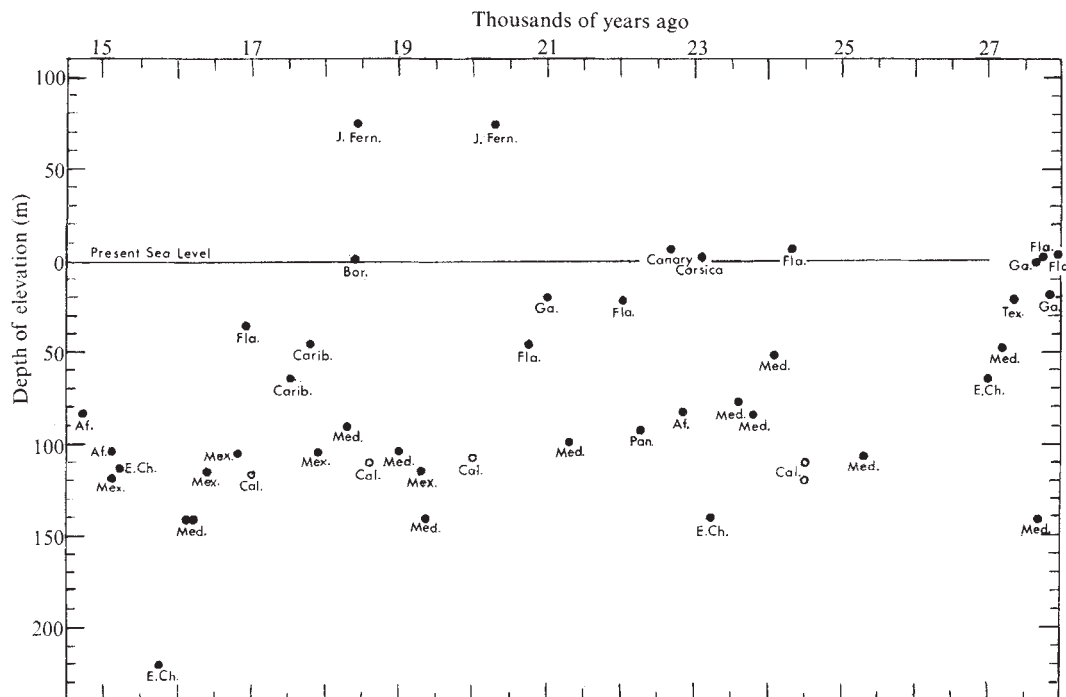
In 1955 Gold calculated that a 30-m uplift of South America would cause the Earth to topple over in this fashion at a rate of 1° per 1,000 yr. The mass of the surface load he thus envisioned would have been less than 1/150th that of the estimated 46×10^6 megatons (ref. 7) of glacial ice in the Ice Age. Fairbridge in 1961 and later gave detailed consideration to the hydrographic importance of polar movement in relation to ancient beaches^{8,9}. Hapgood published his interpretations on polar movement in 1958 and 1970 (ref. 10). Einstein stated that the far-reaching implications of this concept 'electrified' him¹¹.

Figure 1 shows the depths of 47 shoreline samples dated between 14,700 and 28,000 yr ago (Table 1)¹²⁻²⁷. Note that they range over about 300 m, as shown by the scale at the left. Yet we know that the amount of water withdrawn from the sea for the growing glaciers and returned to it with their melting must have been causing a well-ordered fluctuation of sea level throughout the world.

In Fig. 2, the upper panel shows how the dots cluster when the hydrodynamic formula is applied. The seemingly contradictory samples converge to form this pattern when polar movement along the curve in the lower panel is postulated. The curve for sea level was drafted in accommodation of the dot pattern. It purports to show where the sea level would

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Fig. 1 Forty-eight dated shoreline samples from widely scattered regions are plotted with reference to present sea level. Note that the depths and elevations at which they were found range erratically over about 300 m. Samples with similar dates should show similar levels. Compare with Fig. 2.



have stood if the Pole had remained where it is now, leaving the oceans to fluctuate only in response to glacier growth and decline.

Successive remodelling by trial and error disclosed the curves presented here. Even slightly different meridians for polar movement will cause the dots to disperse, and soon they are randomly distributed.

The dots in the lower panel show the polar shift implied by the original depths of the samples, taken in relation to the sea-level curve.

Local uplift or subsidence of the Earth's crust would reduce or exaggerate the role of polar movement. Chance hits could give a spurious semblance of corroboration. Therefore, I have tried to exclude all samples from regions suspected of being geologically unstable. On this basis, Japan, Okinawa, Taiwan, Hawaii, the Mississippi delta (sedimentation), Patagonia and the Mediterranean coast east of Italy are omitted. California samples are shown with hollow circles because of possible tectonism. However, accurate measurements in the region they represent show gradual subsidence at about 2.5 mm yr^{-1} over some five decades^{28,29}. This would come to only 2.5 m in 1,000 yr.

Possible downwarping due to the load of the North American glacier accounts for the omission of samples from the Atlantic coast north of Georgia³⁰⁻³². This would be only about 3,000 km from the centre of glaciation. Also, more complex local movements have been suggested along this coast³³⁻³⁵. For similar reasons we have excluded Pacific Coast samples north of California (the Cordilleran glacier), and European samples north of the Mediterranean, as well as the Himalaya region and Tasmania³⁶, where there was heavy ice build-up.

By this process of elimination, it was hoped that any considerable changes due to polar shift alone might be revealed.

The samples

Equal reliance cannot be placed on all samples related to shorelines. Depending on species, littoral organisms can have lived at (or slumped to) greater depth. An extended habitat range can introduce considerable discrepancy. However, probabilities would make smaller ones more likely.

There are two invariable principles:

Axiom 1: Extended habitat range can only lower the indicated level of the sea, never raise it.

Axiom 2: Where glacial downwarping is suspected, the post-glacial rebound can put a prehistoric shoreline higher than

it would have been without the downwarping, but not lower (except possibly in the forebulge area^{31,37,38}).

Radiocarbon dates also vary in reliability. All specimens that previous writers had suspected of being contaminated, recrystallised, reworked, or redeposited, as well as samples excluded for unspecified faults have been eliminated. Radiocarbon dates have different plus-or-minus spans. Only the 'central' dates were used in plotting the graphs. In general, the analysis strengthened confidence in these dates. One is tempted to suspect that some of the complaints levelled against shoreline samples might better be laid to disregard of the hydrodynamic hypothesis.

The formulation

Change of latitude at the site incident to pole slippage was first determined, using the equation:

$$\sin \text{lat}_2 = \sin \text{lat}_1 \cos c + \cos \text{lat}_1 \sin c \cos A \quad (1)$$

where lat_1 represents the present latitude of the sample site, c the amount of polar shift, and A the difference in longitude between the site and the meridian of polar shift (Greenwich longitude of the axis). Quadrant signs must be observed. South latitudes and east longitudes should be entered as negative.

Second, the difference in sea level for the two latitudes was derived through the equation for an ellipsoid ($x^2/a^2 + y^2/b^2 = 1$), rendered in the following form appropriate to a body with the Earth's dimensions:

$$\text{Difference in sea level (D)} = (\cos [2 \text{lat}_2] - \cos [2 \text{lat}_1]) \times 10,675 \text{ meters.} \quad (2)$$

A positive difference means that the sea level would be higher for lat_2 than for lat_1 , and *vice versa*.

The vertical scale for polar movement is labelled 'factorial' under the assumption that the crust of the Earth would have yielded ellipsoidally at least slightly under the same forces as the oceans. The sea-level changes as derived here reflect only the net difference. The actual polar movement would not be less than the scale figure read in tenths of a degree. It could be considerably greater, depending on the extent of the Earth's response to the stress.

Interpretation

The present analysis tolerates samples that would be grossly deviant in relation to previous sea level curves, yet it yields a reasonably tight dot pattern.

Table 1 Location and characteristics of dated shoreline samples

Yr BP	Location		Depth or elevation (m)	Material	Sample no.	Ref.
14,700	West Africa	4.63°N 6.33°W	— 82	Nodules of calcareous algae	Gif-2139 Core C48	12
15,100	West Africa	5.17°N 4.03°W	—102.5	Nodules of calcareous algae	Gif-2136 Core C1	12
15,100	W. Coast Mexico	21.67°N 106.25°W	~ -118	<i>Hexaplex erythrostomus</i>	LJ-1373	13
15,200	E. China Sea	29.37°N 126.30°E	—112	<i>Ostrea gigas</i>	W-2036	14
15,740	E. China Sea	34.67°N 129.27°E	—219	<i>Macoma calcaria</i>	W2343	14
16,100	W. Mediterranean	43.08°N 5.37°E	—140	Free calcareous algae	MC-352	15
16,200	W. Mediterranean	43.08°N 5.37°E	—140	Free calcareous algae	MC-351	15
~16,400	W. Coast Mexico	22°N 68°W (est.)	~ -115	Shells		16
16,800	W. Coast Mexico	22°N 68°W (est.)	—104	Shells		16
16,920	Florida	29.88°N 80.58°W	— 35	Oolite	L1380	16
17,000	S. California	33.69°N 118.33°W	—116	Calcareous algae	Sample 4 Core 5154	17
17,550	S.E. Caribbean	W. Guiana Shelf	—(55-73)	Reeflike feature		18
17,820	S.E. Caribbean	10.17°N 60.88°W	— 45	Plant remains; twigs	Station 1109	19
~17,900	W. Coast Mexico	22°N 68°W (est.)	~ -104	Shells		16
18,300	W. Mediterranean	42.67°N 3.44°E	— 90.5	Shelly sand	MC-330	15
18,400	Juan Fernandez	33.66°S 78.99°W	ca+ 75	Shells and small organisms	LJ-GAP-60	13
18,400	Borneo	4.33°N 118.50°E	+ 1.5	Coral	N-716	20
18,600	S. California	33.69°N 118.33°W	—108.5	Calcareous algae	Sample 3 Core 5156	17
19,000	W. Mediterranean	43.22°N 4.18°E	—103	Shell fragments (<i>Cyprina islandica</i>)	MC-430	15
19,300	W. Coast Mexico	22.11°N 106.30°W	—114	Conch (<i>Strombus granulosus</i>)	LJ-280	21
19,350	W. Mediterranean	43.08°N 5.37°E	—140	Calcareous algae	MC-439	15
20,000	S. California	33.69°N 118.33°W	—105	Calcareous algae	Sample 1 Core 2981	17
20,000	S. California	33.69°N 118.33°W	—106.7	Calcareous algae	Sample 1 Core 5158	17
20,300	Juan Fernandez	33.66°S 78.99°W	~ + 75	Shells and small organisms	LJ-GAP-61	13
20,730	Florida	29.88°N 80.58°W	— 45	Oolite	L1386	16
21,000	Georgia	31.33°N 80.83°W	— 19	Oyster	Pil 36	16
21,300	W. Mediterranean	42.98°N 3.97°E	— 98.3	Shell (<i>Cyprina islandica</i>)	MC-357	15
22,042	Florida	30.5°N 86.5°W	— 21	Clastic limestone	Core 19	22
22,280	Panama	8.08°N 79.60°W	— 92.5	Mollusc fragments and foraminifera	LJ-GAP-5	13
22,730	Canary Is.	28.86°N 13.85°W	+ 6	<i>Purpura haemastoma</i>	KI-362	23
22,840	West Africa	4.20°N 7.50°W	— 81	Nodules of calcareous algae	Gif-2144	12
23,100	W. Mediterranean (Corsica)	41.45°N 9.02°E	+ 3	Oysters	Gif-1209	24
23,260	E. China Sea	27.10°N 123.50°E	—140	<i>Mactra chinensis Pecten albicans</i>	W2341	14
23,600	W. Mediterranean	43.83°N 8.00°E	— 76.2	Lithothamninae and mollusc shells	Gif-1564	12
23,800	W. Mediterranean	42.53°N 3.22°E	— 83	Marine shells (<i>Venus, Turritella, Mytilus</i>)	MC-167	15
24,100	W. Mediterranean	43.83°N 8.00°E	— 51	Encrusting with mollusc shells	Gif-1565	12
24,330	Florida	29.22°N 81.08°W	+ 7.6	Small broken shells from coquina	UGa-12	25
24,500	S. California	33.69°N 118.33°W	—110.3	Calcareous algae	Sample 2 Core 5156	17
24,500	S. California	33.69°N 118.33°W	—119.5	Calcareous algae	Sample 2 Core 5157	17
25,300	W. Mediterranean	43.22°N 4.18°E	—105.5	Shell fragments	MC-415	15
27,000	E. China Sea	31.00°N 125.52°E	— 64	<i>Corbicula japonica</i>	W2216	14
27,200	W. Mediterranean	42.72°N 3.13°E	— 47	Shell fragments (<i>Chlamys</i> sp.)	MC-250	15
27,380	Texas	27.45°N 97.3°W	— 18.9	<i>Mulinia</i> sp.	Tx-800	26
27,670	Georgia	31.45°N 81.29°W	— 0.5	Oyster shells	UGa-16	25
27,700	W. Mediterranean	43.08°N 5.37°E	—141	Calcareous algae	MC-438	15
27,760	Florida	28.68°N 80.72°W	+ 1.3	Broken shells from coquina	UGa-8	25
27,860	Georgia	31.38°N 80.8°W	— 18	<i>Crassostrea virginica</i>	UGa-231	27
27,960	Florida	29.56°N 81.18°W	+ 3.6	Shells of <i>Mulinia, Tellina, Donax</i> , and <i>Arca</i> from coquina	UGa-11	25

The sea-level curve indicates two glacial advances instead of one as shown by curves derived without taking the hydrodynamic hypothesis into account.

The hump between 17,000 and 22,000 yr ago indicating a return to 'normal' sea level cannot be explained away by extended habitat range, which would only heighten it (Axiom 1). Glacial downwarping might conceivably have occurred in the Mediterranean region (three samples), but 11 samples come from regions that would not be subject to this. This leaves only tectonic displacement. Taking everything into account the hump seems to be statistically interesting.

The dotted righthand portion is considered uncertain because the samples there are not collectively definitive. It is not as easy to plot a plausible curve earlier than 28,000 BP (data variability too great?) or later than 14,700 (polar movement too small?).

The indicated direction of polar movement seems consistent with the dynamic factors involved. The North American ice sheet has been estimated at about 2.74 times the size of the European⁷. Accepting conventional positions for the dynamic centres of the two ice masses, vector analysis predicts that the

resultant force would act along the meridian 65°W. This is only 5° from where the analysis put it.

The curve for polar movement resembles a sine curve, with a twice-recurring period of about 5,600 yr. There is hint of another cycle of similar length with a node at about 29,200 BP (Borneo²⁰ and Bahama¹⁶).

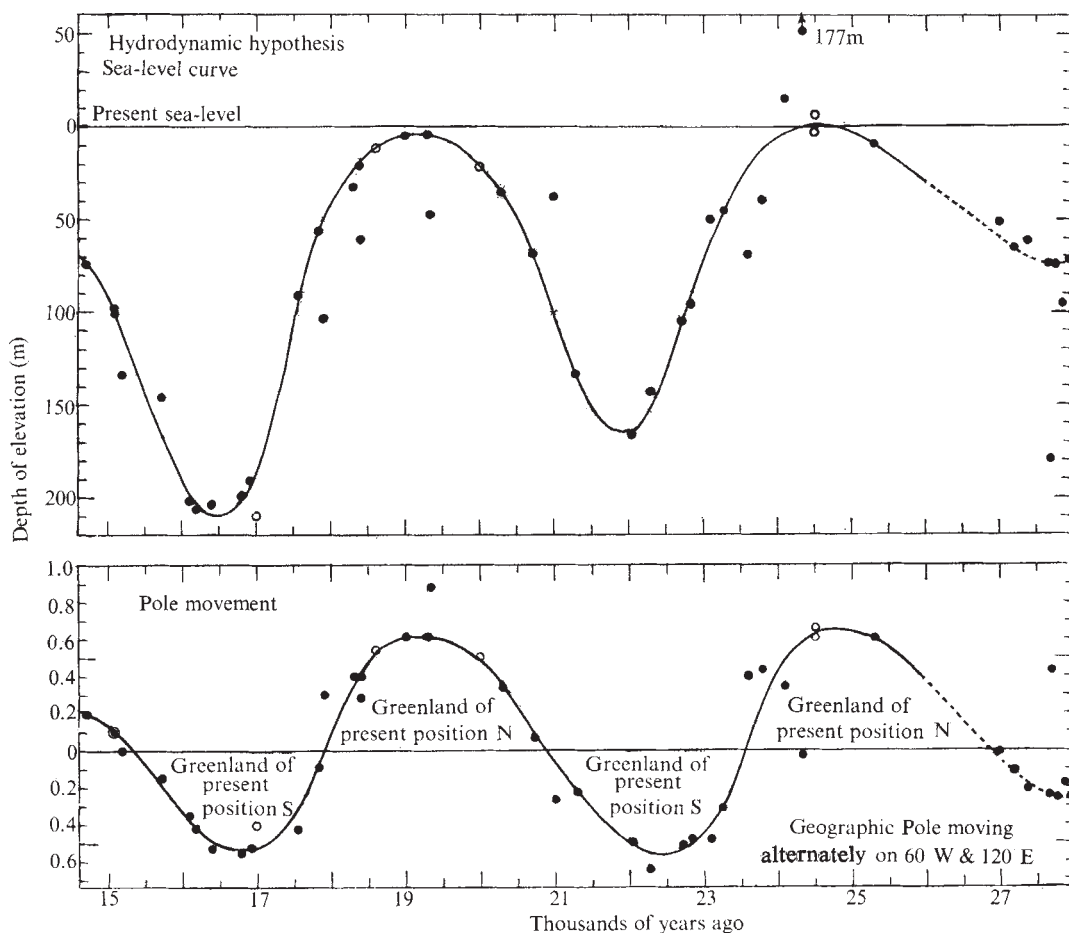
The movement of the Greenland sector southwards begins each time the glaciers begin to grow (as registered by the sea-level curve). This is consistent with the theory that their centrifugal effect caused the polar slippage. The glaciers continued to grow after Greenland started southwards. Perhaps the climatic factor persisted. Or perhaps the high solar reflectivity of the ice masses and their increased altitudes augmented snowfall.

The polar curve is slightly asymmetric. This skewness suggests that the glaciers grew faster than they melted.

The sea-level curve of itself cannot tell the extent to which the Antarctic ice cap may also have fluctuated during this period. The curve registers the quantity of water withdrawn but not its source.

Scepticism may be engendered by two considerations:

Fig. 2 When a shifting Pole is postulated, the individual samples in Fig. 1 congregate along the curve in the upper panel. The lower panel shows the polar movement that would transform the seemingly random dot pattern into this orderly configuration. The polar curve starts downward each time the glaciers begin to grow, as indicated by the falling sea level curve. This is consistent with the theory that the centrifugal force generated by the ice masses would cause the Earth to slip its Pole in this fashion. In the above; $\sin \text{lat}_2 = \sin \text{lat}_1 \cos c + \cos \text{lat}_1 \sin c \cos A$ where c = Pole movement and A = longitude difference. Also Sea level difference = $(\cos [2 \text{lat}_2] - \cos [2 \text{lat}_1]) \times 10,675$ metres.



(1) At the peak of glaciation, the sea level drops further than has been indicated by conventional sea-level curves. (The depth could have been exaggerated by extended habitat range [Axiom 1]. On the other hand, if there were post-glacial rebound, the curve would not show deep enough [Axiom 2].) (2) A mid-glacial recession is indicated which has not been detected in the geological record or revealed by conventional sea-level curves. (The geographical distribution of the samples lessens the likelihood that this hump in the curve could be an error due to glacial downwarping and rebound.)

The likelihood that the findings may express no more than a statistical accident is reduced by the following considerations.

Dynamics: (1) The sinusoidal character of the polar curve is appropriate to a rotating body. (2) The 5,600-yr cycle occurs twice. (3) The direction of polar movement is within 5° of the direction that would be predicted by vector analysis from the relative sizes and positions of the glacial masses.

Climatology. (1) Glaciation is initiated when the regions glaciated reach their highest latitude. Deglaciation begins when they reach their lowest. (The actual amount of polar shift may have been significantly greater than that measured by the factorial scale.) (2) The sea level curve returns twice almost exactly to the present level—unlikely as a coincidence and suggestive of a thermal-dynamic relationship.

The method: (1) The formulation requires concomitant satisfaction of four independent variables, in conjunction with the time scale. None of the variables can dominate the outcome in a statistically representative sampling. (2) On either side of the 'best' meridian, the dot pattern deteriorates progressively, and no other meridian will satisfy. (3) Slight margin of error in interpreting the data would put all but two of the samples directly on the curve. (4) Effort has been made to exclude data that might lead to spurious confirmation through coincidence.

A computer programme for solving the equations rapidly on

the Hewlett-Packard 97 will be sent on request. I thank J. B. Bird, M. H. P. Bott, W. S. Broecker, R. L. Day, R. W. Fairbridge, R. F. Flint, J. Gerver, C. H. Hapgood, S. A. Korff, L. M. Libby, W. F. Libby, P. Melchior, R. B. Mendell, J. D. Milliman, G. Manley, W. H. Munk, and G. S. Vescelius for advice.

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Precambrian geological history and the origin of the Metazoa

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The Hargraves model of the physical world of the Precambrian challenges the traditional view of the geophysical evolution of the Earth. Its implications on the sudden appearance late in the Precambrian of multicellular organisms are discussed.

THE sudden appearance of a diversity of multicellular organisms late in the Precambrian is one of the major enigmas of the fossil record, and constitutes a twofold problem for the palaeontologist. First, it is necessary to explain the timing of the first appearance of metazoan fossils, and, second, the selective factors operating in the evolution of the metazoan phyla must be identified. The origin of the Metazoa has been attributed to changes in ambient oxygen levels or ultraviolet light¹⁻⁵ and/or to organisational changes on the level of organisms or communities⁴⁻⁸, but no single theory adequately explains both the timing and selective factors involved. Previous work has assumed that, with the exception of oxygen and ultraviolet light levels, the physical world in the Precambrian was much as we know it today with oceans and continents, shallow waters and deep. However, Hargraves⁹ has challenged this traditional view of the Precambrian; his model, if substantiated, would have far reaching implications in any discussion of the Precambrian biota. Here I attempt to evaluate some of the implications of Hargraves' model of geophysical evolution of the Earth during the Precambrian for the evolution of the metazoan phyla.

Hargraves' model

Hargraves⁹ proposes that segregation of sialic (continental) crust occurred early in the history of the Earth, and the original surface of the Earth consisted of a thin sialic crust covered by a world ocean about 2.8 km deep. It is assumed that the volume of the hydrosphere has remained essentially constant, but the model is not particularly sensitive to the volume of the oceans.

Hargraves postulates a highly tempestuous early history for the Earth, with continuous subduction and remelting of the sialic crust until the Earth had cooled sufficiently (about 3,500 Myr ago) to allow a continuous, Earth-girdling sialic crust about 8.4 km thick to exist. Mantle convection would then begin to coalesce the sialic crust into a single or a few supercontinents (also see Engel¹⁰). The thickness of this sialic crust would be controlled by the depth of the 750 °C isotherm in the Earth's crust; that is, the depth at which these rocks begin to melt.

If thickness of the sialic crust and supercontinental blocks was controlled by the depth of the 750 °C isotherm, then general emergence of the continental land masses from the ocean could only occur after the 750 °C isotherm had descended to a depth sufficient to allow a column of sialic rock in isostatic equilibrium to reach the ocean surface without melting at its base. General emergence of the continents thus could not have occurred before 3,100–3,600 Myr after the Earth formed (depending on the particular compositional model used); that is, continents could not have emerged from the ocean before ~1,400–900 Myr

ago in the latter part of the Precambrian. But this argument only sets the maximum age of emerged continental land masses; indeed, Hargraves argues on the basis of estimated volumes of sedimentary rocks that actual general emergence of the continents did not occur until late in the Precambrian between 900 and 600 Myr ago.

Hargraves' model has been strongly criticised by Windley¹², who presents the evidence for a more traditional view of the Earth during the Precambrian. But independent evidence supporting Hargraves' basic contention (the existence of high geothermal gradients late in the Precambrian) has recently been presented by Saxena¹³. Using the presence of charnockites, which form only at shallow depths (6–12 km) and in a narrow range of temperatures (800–900 °C), Saxena¹³ concludes that geothermal gradients of 70–100 °C km⁻¹ were present about 1,200 Myr ago, in excellent agreement with Hargraves' estimates. More detailed arguments are given elsewhere^{9,11,13}.

Implications of the model

The primary implication of Hargraves' model is that during the first three-quarters of the Precambrian (approximately equivalent to the Archean and Proterozoic I and II of Schopf¹⁴), shallow water environments (defined here as less than 200 m depth) were very rare, and those that did exist were the result of volcanic or dynamic mountain-building processes and were geologically transitory.

This may seem to contradict our present knowledge of Precambrian biological communities. The fossil record for most of the Precambrian is restricted to algal stromatolites and associated microfossils^{6,7,14,15} which are acknowledged to be of shallow water origin^{6,16-18}. However, these fossils are restricted to a surprisingly few localities (see Schopf¹⁴), and the underlying rock masses have been interpreted as the result of dynamic processes rather than an isostatically stable platform^{9,11,19}.

The implications of this dearth of shallow water environments are profound; for convenience they will be considered first for the planktonic community and then for the benthic community.

Precambrian planktonic communities

Chamberlain and Marland²⁰ have considered some of the implications of Hargraves' model for Precambrian planktonic communities. In particular they point out that without land masses to aid in the generation of deep water upwellings, the Precambrian ocean could be expected to be highly stratified; Precambrian phytoplankters would, by their own activities, deplete the euphotic zone of nutrients.

Table 1 represents the average annual productivity of

Table 1 Productivity in modern oceanic provinces*

Province	Area (km ² × 10 ⁶)	Mean productivity (g C per km ² per yr)	Total productivity (g C per yr)
Open ocean	326	5.0 × 10 ⁷	16.3 × 10 ¹⁵
Coastal zone	36	10.0 × 10 ⁷	3.6 × 10 ¹⁵
Upwellings	3.6	30.0 × 10 ⁷	0.1 × 10 ¹⁵

*After Ryther ref. 21.

modern oceanic regions, and may be used to estimate the productivity of the oceans through the Precambrian. Until continental shoaling, levels of productivity in the world ocean probably corresponded most closely to the productivity of the present open ocean. These figures are undoubtedly an overestimate, as the productivity of modern open ocean areas is strongly dependent on nutrient recycling by herbivores²², and the question of the presence of grazers in Precambrian oceans is moot; however, some modern protozoans do attack blue-green algae²³.

With shoaling of the seas over the continental regions 1,400–900 Myr ago, a tremendous area corresponding to the present coastal zone (depth ≤ 200 m) would be produced, an area approximately equivalent to the present area of the continents. Total productivity of the world ocean would increase by a factor of at least 1.3 and possibly by an even greater amount; depression of productivity in the open waters by a lack of herbivore-mediated nutrient recycling may have made this increase even more dramatic. Local productivity over the shoaling regions would at least double (Table 1).

Precambrian benthic communities

Table 2 shows the distribution of benthic biomass in the present world oceans. Note the strong skew in the distribution of biomass; shallow water environments, which constitute less than 8% of the area of the oceans, contain 83% of the benthic biomass. This large biomass in benthic shallow water communities greatly exceeds what one would expect simply on the basis of differences in primary productivity of the overlying waters (Table 1) and probably reflects a combination of direct grazing of benthic organisms on planktonic primary producers, a reduced loss of detrital material during its fall through the water column, and *in situ* primary production. Organic input to deep bottom sediments in modern waters represents only about 1.7–3.5% of primary production in the overlying waters (depths 1,850 m and 1,230 m respectively²⁸).

During the Archean and Proterozoic I and II (before continental shoaling), the maximum sustainable biomass of the benthos was certainly no greater than 20 g m^{-2} (Table 2). If Menzies²⁶ is correct in his contention that primary production in the surface waters is less important a source of food for abyssal benthos than detrital input from shallow waters (by turbidity currents) in modern oceans, then maximum sustainable benthic biomass for most of the Precambrian was much less than 20 g m^{-2} . Using the figures above for the input of organic carbon to the benthos from primary productivity²⁵ and a trophic efficiency of 15%²¹, benthic communities through the Archean and Proterozoic I and II could only produce 0.13–0.26 g detritivores per m^2 per yr and 0.02–0.04 g carnivores per m^2 per yr (assuming a food chain only two steps long).

During Proterozoic III, with shoaling of continental waters, sustainable benthic biomass would have increased dramatically, probably by at least two orders of magnitude (see Table 2). In addition, for the first time an extensive, illuminated shallow water habitat was created; evidence of metaphtyes dates from about 760 Myr ago⁷.

Origin of the metazoan phyla

The actual origin of the Metazoa is beyond the scope of this paper; following other workers^{7,27–30}, I favour a pelagic planuloid form for the ancestral metazoan. Derivation of the coelenterates from such a planuloid presents no difficulties²⁸, nor does the derivation of the platyhelminths; the characteristics of the latter can be regarded as the result of a planuloid taking up a benthic existence. The time of the origin of the ancestral planuloid and flatworm are unknown and perhaps unknowable; both could be expected to actively avoid the shallow waters with a benthic colloidal silica layer which have yielded most of our knowledge of the Pre-

Table 2 Distribution of benthic biomass in modern oceans*

Depth (m)	Area ($\text{m}^2 \times 10^{12}$)	% Total area	Approximate mean biomass (g m^{-2})	Total biomass ($\text{g} \times 10^{12}$)	Total %
0–200	27.5	7.6	200	5,500	82.6
200–3,000	55.2	15.3	20	1,104	16.6
over 3,000	278.3	77.1	0.2	56	0.8

*After Menzies ref. 24.

cambrian blue-greens^{16,17,31,32} although they may yet be found in shales³³. Probable protozoans (chitinozoans) do occur in the late Precambrian³⁴, which enhances the probability that simple multicellular forms had also evolved.

The origin of the remaining phyla presents more difficulties, as it is necessary to explain the functional significance and selective factors operating on the evolution of the coelom and metamerism, both in fully metameric and oligomeric phyla.

The Mollusca represent a special case in this discussion for, as Stasek³⁵ observes, "Were it not for [the] nearly incidental development of a coelomic space [the pericardial coelom], the Mollusca would today be regarded as an acoelomate phylum. They have escaped that designation on a technicality."

Stasek³⁵ and Salvini-Plawen³⁶ both derive the molluscs from an ancestor approximately on the level of the flatworms. Stasek³⁵ argues that the characteristics of the Mollusca are the consequence of a single evolutionary innovation—the production of a dorsal cuticle by the ancestral 'flatworm,' probably as a protective device. Following this innovation, derivation of the molluscan classes is a consequence of attendant physiological difficulties and specialisation for a variety of life styles opened by this innovation.

Evidence has accumulated in recent years that shows that many of the morphological features of invertebrates are a consequence of mechanical considerations. (For convenient summaries of aspects of the mechanical design of various animal and plant groups see refs 29, 30, 37–39). In particular, Clark²⁹ and Trueman³⁰ convincingly argue that the evolution of a coelomic cavity is closely tied to the acquisition of a burrowing habit in invertebrates.

A coelomic cavity is primitively a hydrostatic skeleton which allows forces generated by the entire body wall musculature to be focused on a small area. There are several secondary advantages to the possession of a coelom (transport of metabolites and excretory products, storage of gametes, protrusion of eversible structures), but the primary function of a coelom during its evolution was that of a hydrostatic skeleton. Subsequent subdivision of the coelom in the oligomeric phyla allowed for regional specialisation of the hydrostatic system²⁹. The importance of a true fluid skeleton in burrowing is indicated by the functionally convergent evolution of the haemocoelic system in molluscs^{29,30}. In this context, the mode of formation of the coelom (enterocoely, schizocoely, gonocoely) is of secondary importance.

Clark²⁹ further argues that, although an undivided coelom is appropriate in a sedentary burrowing animal, metamerism is a functional necessity in animals which are to be capable of sustained burrowing. Segmentation of the coelomic cavity allows localised muscular activity to occur without affecting the whole fluid-muscle system and thus increases the efficiency of local shape changes (as in peristaltic locomotion) without loss of the ability to utilise the entire body wall musculature to produce large forces. The metamerism exhibited by chordates is seen as a parallel but independent development in response to the mechanical constraints of swimming²⁹.

Clark²⁹ and Trueman³⁰ argue that a hydrostatic skeleton (typically a coelom) is a functional necessity for a soft-bodied infaunal organism; secondary modifications allow for the protrusion of a proboscis or tentacular apparatus. Metamerism in the annelid–arthropod line is similarly seen as an adaptation to an infaunal existence by an actively burrowing organism. These developments could only occur in a diverse benthic community where a strong selective pressure to escape epifaunal predators by burrowing existed²⁹.

Precambrian origins of the phyla

Clark's²⁹ and Trueman's³⁰ proposal for the origin of the annelid–arthropod line and the oligomeric phyla and Stasek's³⁴ suggestion for the origin of the Mollusca both assume a diverse benthic fauna on the level of organisation of flatworms. Furthermore, it is assumed that some of these organisms were specialised for a predatory existence on the suspension or detritus feeding members of the fauna. Given these two assumptions, which are reasonable, diversification of the fauna into the stem lines for the remaining phyla could be expected.

In this context, Hargraves'⁹ model of the geophysical evolution of the Earth is particularly significant. Before the shoaling of the ocean over the continental land mass(es), not only was primary production in the oceans low but, because of the extreme depth of the water column, detrital input to the benthos and sustainable benthic biomass were both extremely low. With continental shoaling, several synergistic effects would occur simultaneously. Increased nutrient recycling would at least double the primary production in the shallowing waters, while dissolved oxygen levels simultaneously increased; both factors would favour the evolution of planktonic planuloid predators if this had not already occurred and the diversification of the planktonic fauna if the planuloids had already evolved. Detrital input to the benthos would increase by an order of magnitude, while, for the first time, an extensive illuminated shallow water habitat with significant *in situ* primary production would be produced. If a benthic planuloid/flatworm had not already evolved, this wealth of available organic material in itself would be sufficient to answer the question⁷ of "what 'caused' these early metazoans to 'discover the bottom' . . ." Sustainable benthic biomass would increase by at least two orders of magnitude, and, with such a rich resource of detritivores and herbivores, the evolution of benthic predators would quickly follow. Some of these organisms could be expected to evolve a dorsal cuticle as an antipredator device; the diversity of the Mollusca would follow³⁵. Other forms would escape predation pressure by taking up an infaunal habit, yielding detritus or suspension feeding coelomate worms and suspension feeding lophophorates, sustaining themselves on the rich rain of phytoplankton and detritus from above. (Also see refs 40, 41 for compatible discussions of the evolution of lophophorates in this ecological context.) Some of these coelomate worms could then be expected to begin an active and sustained burrowing habit to exploit the available detritus more than a worm's-length away from a fixed burrow; such a habit would result^{29,30} in the metamERICALLY segmented annelids which had evolved by the time the Ediacara fauna was preserved^{42,43}. Evolution of parapodia and intrinsic parapodial musculature would set the stage for the evolution of the arthropods; this also must have occurred before ~700 Myr ago given the presence of annelids with aciculary-supported parapodia⁴² and of arthropods⁴³ in the Ediacara fauna.

According to this model, before ~1,400–900 Myr ago the evolution of a diverse benthic fauna and the higher invertebrate phyla was impossible, the environmental setting (through geophysical processes) being incompatible with such an accomplishment. Sometime between 1,400 and

700 Myr ago, with shoaling of the waters over the continental land mass(es), such a development becomes inevitable. This reconstruction is supported by the trace fossils of locomotory trails (supposedly molluscan) which predate the Ediacara fauna⁴⁴, burrow structures about 800 Myr old⁴⁴, and the numerous burrows and trails contemporaneous with the Ediacara fauna^{44–47}. The observed simultaneous origin of metaphytes and metazoans^{7,8,14} can similarly be rationalised in light of the control an extensive shallow water habitat would exert on their evolution. The interval postulated here for the evolution of the metazoan phyla is considerably shorter than has been suggested by Durham⁴⁸, but agrees well with other estimates^{41,47}.

Discussion

The major point which I hope to have demonstrated is the control of the evolution of higher metazoans exerted by the availability of an extensive shallow water environment; an extensive shallow water habitat is a precondition to the evolution of the higher metazoan taxa.

This reconstruction should not be viewed as supplanting previous theories on the origin of the metazoan phyla, but rather as complementing them. The positive feedback between trophic levels as diversity in each increases obviously follows Stanley's^{8,47} ecological arguments for the evolution of the metazoans, although specific details of Stanley's reconstruction have been negated by recent work on observed eukaryotic diversity in the Precambrian^{32,49,50}. Similarly, Berkner and Marshall's^{1,2} and Rhoads and Morse's⁵¹ arguments on the control of oxygen level on metazoan evolution complement the present model. The presence of collagen is a functional necessity for coelenterates and burrowing organisms^{29,39} and collagen must have been present in the Ediacara fauna, but this does not negate Towe's⁵ discussion of the potentially high metabolic expense of collagen production and shell secretion in Precambrian faunas. The phylogeny and selective factors involved in the evolution of the metazoan phyla agrees with Valentine's^{41,43} reconstruction.

It would be expected that evolving Precambrian faunas would consist of animals small in size, not only because of the expense of collagen production and associated locomotory difficulties⁵, but also because of the amelioration of physiological problems in the absence of highly evolved organ systems that small size *per se* entails⁵³. Further studies on the evolution of metazoan diversity in the Precambrian might then more usefully concentrate on the diversity and structure of small trace fossils⁵¹, rather than a continued search for body fossils which, by the very nature of the animals, were unlikely to be preserved. The ancestral protomollusc seems the most likely of these early forms to be preserved, but recognition may prove difficult. Operationally, the most likely test of this model will be through correlation of trace fossils with changing average water depth in the late Precambrian.

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Nitric acid cycle process for extracting thermal energy from low-level heat sources

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A process for recovering thermal energy from a low-level heat source has been devised. The process utilises the heat generated when nitric acid and water are mixed. The temperature rise in a single-effect unit is about 20 °C, but a higher temperature is attained in a multiple-effect unit.

ENORMOUS amounts of solar energy are stored in the tropical oceans. The first attempt to recover this 'solar sea energy' from the tropical oceans, using the temperature difference between the warm surface sea-water and the cold deep sea-water, was made by Claude¹ in 1930. He succeeded in generating 10-22 kW power in a simple plant off Cuba, but the solar sea power at that time was too costly to compete with the power produced by cheap fossil fuels.

There is now great concern about the fact that oil and gas, which provide most of our energy needs, are rapidly being depleted. Considerable efforts are being made to find alternative energy sources, and interest in ocean thermal energy has therefore increased.

Claude's open-Rankine cycle process in which low-density water vapour drives a turbine generator, however, looks impracticable to many engineers today, and much attention is being paid to a closed-Rankine cycle process using a working fluid such as ammonia or propane. Recent developments of the process have been extensively reviewed by Dugger².

As well as for conversion into electrical energy, the temperature difference may be utilised in other processes such as desalination. Sheth, Peck and Wasan³ proposed a desalination process in which the temperature difference is enhanced by solar heating of the warm surface sea-water.

There has been little research, however, into methods of up-grading low-level heat. Here we describe a process for the conversion of low-level solar sea energy into a higher-level heat, by using temperature difference available in the tropical oceans. The process could also be applied to warm waste water from industry and cold water from lake or river.

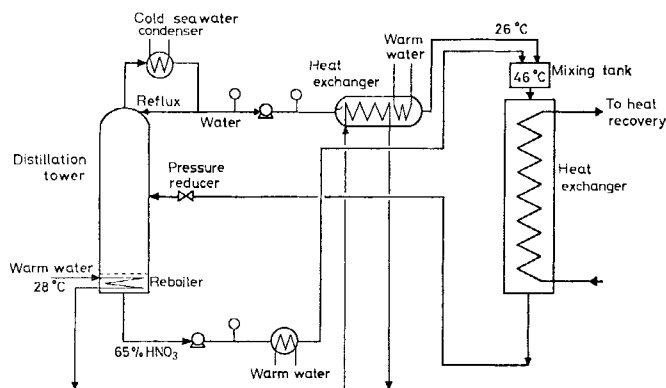
Nitric acid cycle process in single- and multiple-effect units

The process capitalises on the heat generated when nitric acid and water are mixed. When 1 kg of 65% nitric acid is diluted with 0.4-0.8 kg of water (or water containing 0-1% HNO₃), the temperature rises by 20-21 °C. The temperature increase is not small compared to the temperature difference between the warm surface seawater and cold deep seawater available for the conventional ocean thermal energy conversion.

Nitric acid forms a maximum boiling point azeotrope with water. Under atmosphere the azeotrope is at a concentration of 68.8%, boiling at 122 °C. According to our measurements, the azeotrope at 9 mmHg is about 65% nitric acid, boiling at about 25 °C. Water boils at this pressure at 9.7 °C.

The mixture of nitric acid and water is, therefore, separated back into about 65% nitric acid and water in a distillation column operated under the low pressure. Warm surface seawater at about 28 °C is used as the heating medium in the distillation reboiler and cold seawater at

Fig. 1 Heat recovery in a single-effect unit.



5–7 °C as the cooling medium in the overhead condenser.

Figure 1 shows the equipment flow diagram, comprising primarily the distillation column and a tank for mixing nitric acid and water. The distillate (water) and the bottom liquid (~65% nitric acid) from the distillation column are pumped up to about 50 mmHg (or higher) and heated in a heat exchanger using warm seawater as the heating fluid. The liquids are pressurised before mixing in order to keep the mixture in the tank in a liquid state.

If the warm water enters the heat exchanger at 28 °C and heats the two liquids, nitric acid and water, to 26 °C, and if the temperature rise is 20 °C (this occurs when mixing 1 kg of 65% nitric acid and 0.4 kg of water), the mixture in the tank attains 46 °C. The heat recovery efficiency, defined as the difference in enthalpy of the mixture between 46 °C and the temperature of the warm water (28 °C) divided by the heat supplied from the warm water to the reboiler, is 5.0–5.3%, depending on the water-to-nitric acid mixing ratio, as shown in Table 1.

A higher temperature is attained in a unit installed with several effects. In a four-effect unit as shown in Fig. 2, for instance, the nitric acid and the water from the second distillation column are heated to 44 °C by 46 °C mixture from the first tank. The mixture in the second tank then reaches 64 °C because of the 20 °C temperature increase. Similarly, the third and the fourth mixing tanks are, respectively, at 82 °C and 100 °C. The heat recovered is again the enthalpy of the mixture from the fourth tank at 100 °C minus that at 28 °C. The recovery efficiency is the recovered heat divided by the heat supplied to the four reboilers.

Table 1 Heat recovery efficiencies and temperatures attained in single- and multiple-effect units

Water (kg) to be mixed with 1 kg 65% nitric acid	Heat recovery efficiency (%)	Temperature attained (°C)					
		No. of effects:	1	2	3	4	5
0.3	5.3	44	59	75	90	106	
0.4	5.2	46	64	82	100	118	
0.5	5.0	47	66	85	104	123	

The recovery efficiency is not affected by the number of the effects. This is the advantage of the cycle process making use of the heat of dilution. The temperature attained in the multiple-effect unit is also illustrated in Table 1.

In the multiple-effect unit, multiple tanks for mixing nitric acid and water have to be installed, but multiple distillation columns are not necessary. One large tower can be used instead of several independent columns.

Split-column system

If instead of each single distillation tower, two or more columns are operated under different low pressures, larger temperature differences can be used as driving forces for heating reboilers.

Table 2 Comparison in reboiler characteristics between single column and split-column

	Single (nonsplit) column	Double split-column					
T_1 (°C)	28	28	28	28	28	28	28
T_2 (°C)	26	26	25	24	23	22	21
M (kg h ⁻¹)	7,430	14,450	9,630	7,230	5,780	4,820	4,130
ΔT (°C)	1.8	7.0	6.4	5.8	5.1	4.3	3.4
A (m ²)	16.5	8.3	9.0	10.0	11.3	13.4	17.0

Mixing ratio: 0.4 kg water to 1 kg 65% nitric acid. Overall heat transfer coefficient: 500 kcal m⁻² h⁻¹ deg. C⁻¹.

Calculation basis: 860 kcal h⁻¹ (1 kw thermal) heat generation in mixing tank.

T_1 , warm water inlet temperature; A , heat transfer area; T_2 , warm water outlet temperature; M , quantity of required warm water.

Figure 3 illustrates the two columns operated, respectively, at 7 and 11 mmHg. Let us assume that the liquids are boiling at 20 °C in both reboilers. This occurs when the liquid in the 7 mmHg reboiler is about 65% nitric acid and that in the 11 mmHg reboiler is about 30%. The mixture of 1 kg 65% nitric acid from the lower-pressure column and 0.4 kg water from the 11 mmHg column returns to the lower-pressure column.

If the warm water enters the reboiler at 28 °C and leaves at 26 °C, the logarithmic mean temperature difference between the warm water and the boiling liquid is $\Delta T = 7.0$ °C in either reboiler. Note that the temperature difference in the single non-split tower reboiler is $\Delta T = 1.8$ °C in the same conditions of warm water temperature. In the split-column the heat recovery efficiency becomes lower, but the total heat transfer area may be considerably reduced.

Table 2 shows a comparison of the reboiler characteristics of a single non-split distillation column and a double-split column. Because of the low-pressure distillation, a falling film type of multiple-tube evaporator is considered suitable for the reboiler.

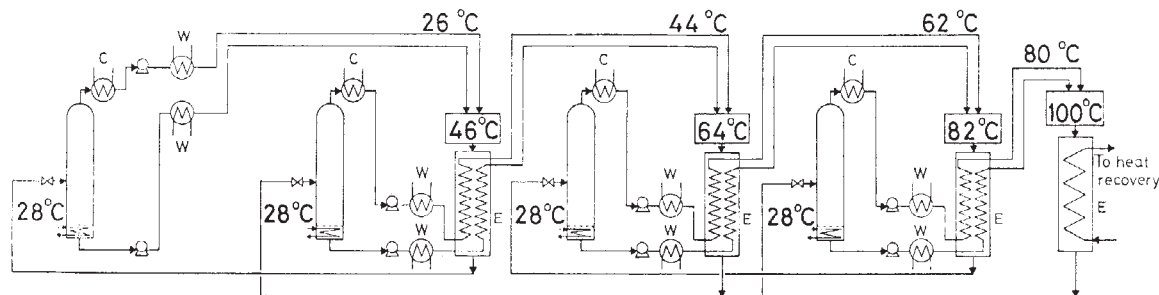
Useful-heat gain

In the conventional ocean thermal energy conversion plant, where electric energy is generated on the closed-Rankine cycle, the largest and most costly elements are the evaporators for a working fluid and the next largest are the condensers in which the vapour of the working fluid is condensed. This is because the available temperature differences are small.

Similarly, the reboilers and overhead condensers of a nitric acid cycle plant would be very large and costly elements. In this respect, the heat transfer areas of the reboilers estimated in Table 2 (assuming 500 kcal m⁻² h⁻¹ deg. C⁻¹ overall heat transfer coefficient) will give a notion of the plant size.

In the calculation of heat recovery, no heat losses are assumed in the transfer lines, mixing tanks and elsewhere. In steady operation conditions the distillation columns are

Fig. 2 Heat recovery in a multiple-effect unit. C, condenser; E, heat exchanger; W, warm water heater.



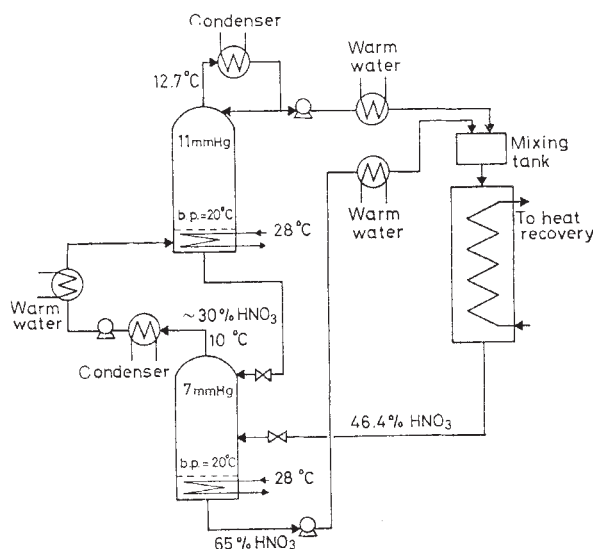


Fig. 3 Diagram for split-column system.

kept at low pressure without any evacuation power requirement, but the power is needed at the start-up time. Pumps for circulating the warm and cold water streams and the process stream are also not taken into consideration.

When these aspects are considered, the efficiency, or overall useful heat gain, is reduced. For accurate estimation of efficiency more information is needed on overall heat

transfer coefficient values of the reboilers, overhead condensers and heat exchangers, temperatures of the warm and cold waters, type of the distillation columns and so on.

However, the situation is again similar to the conventional ocean thermal energy conversion plant. Dugger² points out that the theoretical efficiency of the closed-Rankine cycle, defined as the working fluid's enthalpy change through the turbine divided by its enthalpy change through the evaporator, operated with ammonia on the temperature difference of 20 °C is 3.3%, but it drops, because of the pumping power and efficiencies of the pumps, turbines and generators, to a net value near 2.5%.

Judging from this about 4% overall useful heat gain will be expected for the nitric acid cycle process. The efficiency is low, but it should be remembered that even a theoretical efficiency of Carnot cycle is not high; Carnot cycle efficiency operating between 28 °C and 6 °C, for instance, is 7.3%.

Although its economic feasibility is not yet established, we expect that the process of extracting pollution-free thermal energy of a higher temperature from solar sea energy or waste heat will become a promising candidate as a new energy source.

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Plasmid incompatibility: cloning analysis of an *incFII* determinant of R6-5

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SalI and *PstI* restriction endonuclease-generated DNA fragments that specify an *FII*-type incompatibility function (*incFII*) of the low copy number antibiotic resistance plasmid R6-5 have been cloned in the high copy number *pBR322* plasmid vector. A 1-kilobase DNA sequence that contains this *incFII* determinant has been identified and is shown to have coordinates of 95.5 and 96.5 kilobases on the R6-5 plasmid physical map. Expression of incompatibility by the cloned *PstI* fragment depends on its orientation within the vector molecule. The behaviour of *pBR322-incFII* hybrid plasmids suggests that plasmid replication control is not the only mechanism that can cause incompatibility between two plasmids.

PLASMIDS are autonomous extrachromosomal genetic elements that code for a variety of functions, such as resistance to antibiotics and the production of toxins¹. Although such functions are not ordinarily essential for normal growth of host bacteria, most naturally occurring plasmids are stably maintained and inherited. This suggests the existence of specific mechanisms which ensure that, in dividing cells, plasmid molecules double once in number during every cell generation and that, at cell division, they are accurately partitioned between daughter bacteria.

Mechanisms for the transfer of DNA among bacteria are widespread¹, and the accumulation of several different plasmids in a single bacterial clone is often observed. If two distinct plasmids can co-exist and be stably co-inherited in individual dividing bacteria, they are termed compatible, while if they cannot be stably co-inherited, they are termed incompatible². Incompatible plasmids usually have extensive sequence homology and exhibit similar replication properties³⁻⁵. This finding has enabled plasmid incompatibility reactions to be utilised widely as tests of plasmid relatedness for classification purposes, and for epidemiological studies of the spread of clinically important plasmids in communities of animals and humans⁶. It should be noted, however, that extensive sequence homology is not always correlated with incompatibility between two plasmids⁷. An analysis of the mechanisms underlying the phenomenon of incompatibility is therefore of some interest both for interpretation of incompatibility tests and, more importantly, for an understanding of the basic process of plasmid inheritance in dividing bacteria.

In order to identify the individual functions and analyse the specific mechanisms involved in plasmid incompatibility, we have begun to clone small fragments of plasmid DNA that may be involved in the phenomenon. We report here the construction of two hybrid plasmids composed of the high copy number vector *pBR322* and DNA fragments that express an *FII*-type incompatibility, and that were derived from the *FII*-antibiotic resistance plasmid R6-5. These *pBR322-incFII* hybrid plasmids

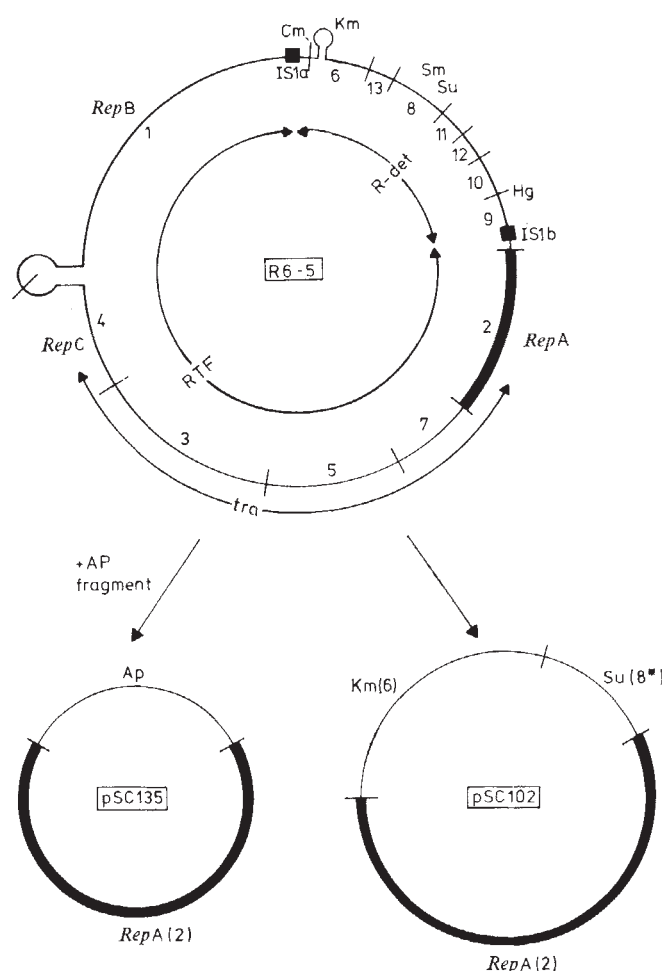


Fig. 1 The R6-5 plasmid and its 'mini' derivatives pSC135 and pSC102. The physical map of the R6-5 plasmid (molecular length 98.5 kilobases, ref. 17) that is depicted is essentially that described by Sharp *et al.*⁷. The locations of *EcoRI* endonuclease cleavage sites in R6-5 have been determined by Timmis *et al.*¹⁰ and are similar to those described for R100³⁰ and NR1³⁰. The pSC135 plasmid was obtained by *in vitro* linkage of R6-5 *EcoRI* fragment 2 (molecular length 12.7 kilobases; also called the *repA* *EcoRI* fragment of R6-5, refs. 8, 10, heavy lines) to an *EcoRI* DNA fragment from *Staphylococcus aureus* plasmid p1258 which specifies resistance to ampicillin and which is incapable of self-replication in *E. coli*⁸. The pSC102 plasmid was obtained by *in vitro* linkage of *EcoRI*-generated DNA fragments of the R6-5 plasmid¹³ and contains in addition to R6-5 *EcoRI* fragment 2, two other fragments of R6-5 that specify resistance to kanamycin (Km; fragment 6) and sulphonamide (Su). Although *EcoRI* fragments 2 and 6 of pSC102 seem to be genuine fragments of R6-5, the Su fragment, designated 8*, is not, and seems to be R6-5 fragment 8 in which a deletion has occurred causing loss of the streptomycin resistance that is normally specified by this fragment¹⁰.

should be of considerable value in the analysis of the phenomenon of plasmid incompatibility and have already provided some evidence implicating the involvement of a mechanism additional to that of replication control.

Location of an FII-type incompatibility determinant on plasmid R6-5

Figure 1 shows a physical map of the antibiotic resistance plasmid R6-5 which exhibits FII-type incompatibility. In previous communications we showed that all plasmid-specified functions essential for controlled autonomous replication and FII-incompatibility were clustered in a small region of the R6-5 molecule and were contained on *EcoRI* fragment 2 of this plasmid⁸⁻¹⁰. We assume that functions essential for controlled

autonomous replication include a specific DNA sequence at which initiation of DNA replication occurs (an origin of replication), a function that regulates the frequency of initiation of replication (copy control function), and an essential replication activity, of unknown function, analogous to the *repA* locus of plasmid R100 (refs. 11, 12). The existence of this latter function is indicated by the isolation of 'mini R6-5' plasmids that are temperature sensitive for replication (K.N.T. and F. Cabello, unpublished). Other functions, as yet unidentified, may also be essential. Two mini R6-5 plasmids, pSC102 (ref. 13) and pSC135 (ref. 8) (Fig. 1), that contain the *EcoRI* fragment 2, have been described and used in our investigations.

Figure 2 shows that *SalI* and *PstI* restriction endonucleases, respectively, cleave the pSC102 plasmid into three and ten DNA fragments greater than 0.3 kilobase pairs in size. Using the pBR322 plasmid cloning vector¹⁴, we have cloned all of the *PstI* and two of the three *SalI*-generated DNA fragments of the pSC102 plasmid¹⁵. Examples of recombinant plasmids containing individual DNA fragments of pSC102 are shown in Fig. 2. Furthermore, by digestion of pSC102 plasmid DNA with *PstI*, followed by ligation with DNA ligase and transformation of *Escherichia coli*, we were able to isolate several mini pSC102 plasmids which are capable of autonomous replication and which specify resistance to kanamycin (for example pKT071). All *PstI* recombinant plasmids capable of autonomous replication that were constructed without inclusion of a cloning vector contain at least three specific *PstI*-generated DNA fragments of the pSC102 plasmid: fragments 1, 4 and 6. Fragment 1 specifies resistance to kanamycin and, because it carries only 0.2 kilobases of the IS1 distal terminal region of the *EcoRI* fragment 2 of R6-5 (Fig. 3 and ref. 15), is assumed not to code for any functions essential for replication. On the other hand, *PstI* fragments 4 and 6 both seem to be obligatory for autonomous replication of the pSC102 plasmid. First, they are adjacent in the parent molecule (Fig. 3), and are present in the same order and orientation with respect to one another in the mini pSC102 plasmid as they are in the parent pSC102 (ref. 15). Second, *PstI* fragment 4 has recently been shown to contain the origin of replication of the pSC102 plasmid¹⁶. Third, the replication properties of pBR322 hybrid plasmids containing pSC102 *PstI* fragment 4 or 6 or pSC102 *SalI* fragment 2 (which contains most of *PstI* fragment 6) are typical of the pBR322 rather than the pSC102 plasmid.

The pSC102 plasmid does not require high cellular levels of DNA polymerase I for replication, whereas the pBR322 cloning vector does (Table 1). Consequently, although both plasmids are stably inherited in mutant bacteria that produce a temperature-sensitive DNA polymerase I when such bacteria are cultured at a permissive temperature (32 °C in the case of strain KT446), only the pSC102 plasmid replicates in bacteria cultured at the non-permissive temperature (42 °C). Thus, pBR322 hybrid plasmids containing all DNA sequences of pSC102 that are sufficient for pSC102 plasmid replication should be able to replicate at 42 °C in KT446, whereas those hybrid plasmids incapable of utilising the pSC102 replication system should not.

Table 1 shows that hybrid plasmids composed of pBR322 and either pSC102 *PstI* fragment 4 or 6 or pSC102 *SalI* fragment 2 do not replicate at 42 °C in KT446, whereas the mini pSC102 plasmid pKTO71 that contains both *PstI* fragments 4 and 6 replicates stably at this temperature. Thus, hybrid molecules containing *PstI* fragment 4, *PstI* fragment 6 or *SalI* fragment 2 of the pSC102 plasmid do not carry all functions and DNA sequences required for the autonomous replication of this plasmid, and must therefore replicate by means of their pBR322 vector-specified replication system. Consistent with this conclusion is the finding that the *PstI*-4, *PstI*-6, and *SalI*-2 hybrid plasmids are maintained at high cellular levels typical of the pBR322 vector, whereas the mini pSC102 plasmid is maintained at a low cellular level typical of the pSC102 parent (Table 1). This result demonstrates that these pBR322 hybrid plasmids are subject to the replication control mechanism specified by pBR322 and not to that specified by pSC102.

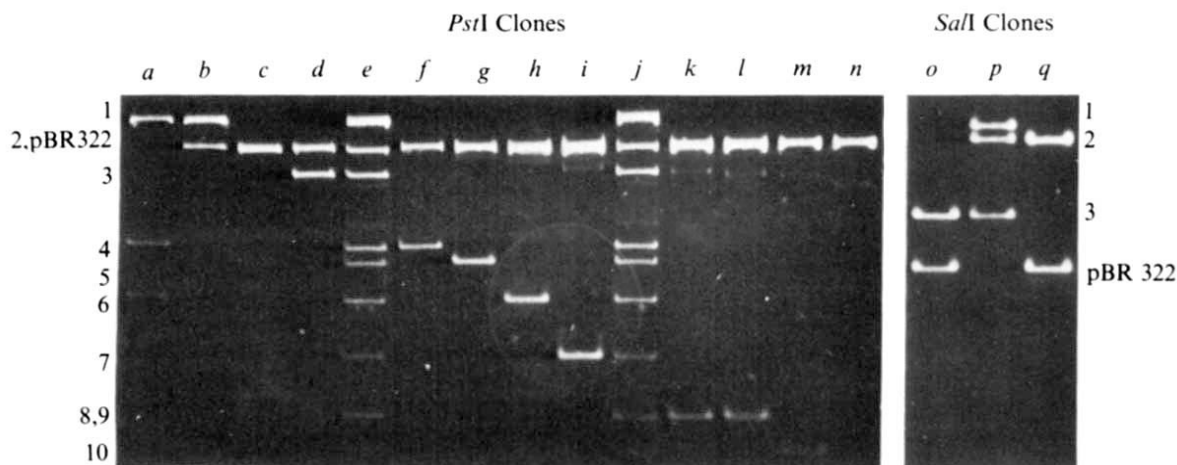


Fig. 2 Cloning of *Pst*I and *Sal*I endonuclease-generated DNA fragments of the pSC102 plasmid with the pBR322 vector. Cloning of individual *Pst*I and *Sal*I generated DNA fragments of the pSC102 plasmid was carried out using conventional cloning procedures¹⁵ and utilising insertional inactivation^{26,31} of the ampicillin (*Pst*I cloning) and tetracycline (*Sal*I cloning) resistance determinants of the pBR322 cloning vector¹⁴. The mini pSC102 plasmid, pKT071, was obtained by ligation of *Pst*I-cleaved pSC102 plasmid DNA followed by transformation in which selection was made for kanamycin plasmids. The miniplasmids obtained by this method do not contain pBR322 vector DNA. Hybrid plasmids were cleaved with the specific endonucleases used for their construction and subjected to electrophoresis through agarose gels to identify the cloned fragments. Samples of 0.5 µg of circular plasmid DNA were digested with 1 unit of restriction endonuclease in 50 µl volume reactions at 37 °C for 60 min. Reaction solutions contained 20 mM Tris-HCl, pH 7.6, and 10 mM MgCl₂ for *Pst*I digests, and 8 mM Tris-HCl, pH 7.6, 6 mM MgCl₂, 0.2 mM EDTA, 150 mM NaCl and 50 µg ml⁻¹ of bovine serum albumin for *Sal*I digests. Enzyme reactions were terminated by the addition of 10 µl of a solution of 60% sucrose containing 1% sodium dodecyl sulphate, and 0.01% bromophenol blue. Digested DNAs were analysed by electrophoresis at 50 mA and 80 V for about 150 min through 0.8% or 1% agarose slab gels containing 90 mM Tris, pH 8.3, 90 mM boric acid, 2.5 mM EDTA and 0.5 µg ml⁻¹ ethidium bromide. The gels were subsequently illuminated with short wave UV light, and the fluorescent DNA bands were photographed with a Polaroid camera fitted with a Kodak Wratten no. 16 orange gelatin filter. a, pKT071 (mini pSC102; *Pst*I-1+4+6; no pBR322); b, pKT029 (*Pst*I-1); c, pKT032 (*Pst*I-2); d, pKT036 (*Pst*I-3); e, *Pst*I-digested pSC102 reference; f, pKT039 (*Pst*I-4); g, pKT040 (*Pst*I-5); h, pKT043 (*Pst*I-6); i, pKT046 (*Pst*I-7); j, *Pst*I-digested pSC102 reference; k, pKT047 (*Pst*I-8); l, pKT052 (*Pst*I-9); m, pKT054 (*Pst*I-10); n, *Pst*I-digested pBR322 reference; o, pKT085 (*Sal*I-3); p, *Sal*I-digested pSC102 reference; q, pKT081 (*Sal*I-2).

Preliminary experiments demonstrated that the only DNA fragments cloned from pSC102 which could provoke an incompatibility reaction with the pSC102 plasmid and parent R6-5 or related R100-1 plasmids were *Sal*I fragment 2 and *Pst*I fragment 6. These two restriction enzyme fragments have in common a DNA sequence of approximately 1 kilobase (Fig. 3) that has one *Pst*I and one *Sal*I terminus. As expected, mini pSC102 plasmids, all of which contain *Pst*I fragment 6, also express incompatibility towards R6-5-like plasmids. This result indicates that an FII incompatibility determinant of the R6-5 plasmid is contained within a 1-kilobase segment of DNA which has one terminus delineated by a *Sal*I cleavage site located 3 kilobases from the R-determinant proximal end of the *repA* *Eco*RI fragment of R6-5. This DNA segment therefore has kilobase coordinates of 95.5 and 96.5 on the R6-5 physical map of Hu *et al.*¹⁷. Recently, a *Pst*I fragment carrying an incompatibility determinant of the FII incompatibility plasmid R1-19 was cloned in the pBR322 plasmid vector (R. Kollek and W. Goebel, personal communication). This DNA fragment is not essential for autonomous replication of mini R1-19 plasmids and has been shown to be located closer to the proximal ISI than is the *Pst*I-6 fragment of the R6-5 plasmid.

It should be noted that the *incFII* determinant identified here is distinct from the R6-5 origin of replication which has been shown to be located on *Pst*I fragment 4 (ref. 16) and which does not exhibit detectable incompatibility towards the pSC102 plasmid. This finding is consistent with a similar result recently obtained with the F plasmid^{18,19}, and indicates that the origin of plasmid replication, although it may participate in, is not itself responsible for, incompatibility reactions.

Structure of *Pst*I-6 and *Sal*I-2 plasmids

Although one *Pst*I-6 hybrid plasmid, pKT043, was found to be incompatible with R6-5 and mini R6-5 derivatives, a second *Pst*I-6 hybrid, pKT042, was found to be compatible. This result suggested that the orientation of the *Pst*I-6 fragment relative to DNA sequences surrounding the *Pst*I site of the pBR322 vector might be important for expression of the

incFII phenotype in the hybrid plasmids. Analysis of the physical structure of the two *Pst*I-6 hybrid plasmids by cleavage with restriction endonucleases (Fig. 4a) confirmed that this is the case. Figure 4b shows the structures of the two *Pst*I-6 and the *Sal*I-2 plasmids. The arrows indicate the direction of the proximal ISI sequence in the complete R6-5 molecule (Fig. 1).

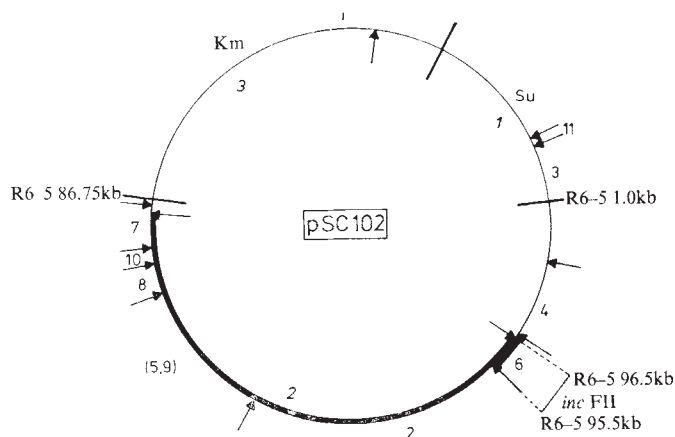


Fig. 3 Location of R6-5 incompatibility locus on the pSC102 plasmid map. A *Pst*I and *Sal*I restriction endonuclease cleavage map of the pSC102 plasmid was constructed by conventional means using double digests with a variety of different restriction endonucleases. A more detailed map and a description of its derivation will be published elsewhere¹⁵. The ISI-proximal *Eco*RI cleavage site of the *repA* *Eco*RI fragment (Fig. 1) has the R6-5 coordinate 1.0 kilobase (ref. 8) whereas its ISI-distal *Eco*RI cleavage site has been given an R6-5 coordinate of 86.75 kilobases, based on a revised size estimate of 12.75 kilobases for this fragment¹⁵. The *incFII*-carrying restriction fragments are indicated by heavy lines. The dotted lines indicate the 1-kilobase R6-5 sequence common to the two *incFII* hybrid plasmids, *Sal*I-2 and *Pst*I-6, which has one *Sal*I terminus and one *Pst*I terminus. The ISI-proximal terminus of fragment *Sal*I-2 is 3.0 kilobases from the ISI-proximal terminus of the *repA* *Eco*RI fragment and therefore has the R6-5 coordinate 96.5 kilobases.

It may be seen that in the two *inc*⁺ hybrid plasmids the R6-5 sequences are (1) inserted in different locations in the pBR322 vector and (2) inserted in opposite orientations with respect to one another. Thus the precise site of the insertion within the vector (for example, with respect to its origin of replication) and the orientation of the inserted fragment with respect to the overall orientation of the vector do not seem to be important for the expression of incompatibility. However, the orientation of cloned fragments inserted at a particular cleavage site, in this case the *Pst*I cleavage site, is crucial for expression of *inc*FII, which presumably depends on transcriptional activity originating from a pBR322 promoter. The incompatibility function coded by this fragment is therefore not, or is not only, a specific DNA recognition sequence, but rather is probably a plasmid protein. This suggestion is supported by recent studies of the proteins synthesised by plasmid-containing minicells in which a difference in the proteins directed by the two *Pst*I-6 hybrid plasmids was demonstrated¹⁵.

Incompatibility reactions of hybrid plasmids that specify *inc*FII

An analysis of incompatibility reactions provoked by the newly constructed hybrid plasmids is shown in Table 2. For these reactions, bacteria of *E. coli* K-12 carrying one member of the indicated plasmid pair were transformed with plasmid DNA of the other member. After transformation, bacteria were spread on nutrient agar plates containing two antibiotics appropriate for selection of clones carrying both plasmids. Unpurified but well isolated transformant colonies were suspended in L-broth and diluted, and the diluted suspensions plated on nutrient agar to obtain single colonies. These were subsequently transferred to antibiotic-containing nutrient agar

plates which would permit detection of each of the component plasmids that was initially present. Because both types of mini-plasmids containing only R6-5 DNA sequences (that is, pSC102 and pKT071) are, like R6-5, resistant to kanamycin, it is not possible to distinguish them by phenotype from R6-5. The incompatibility properties of these mini R6-5 plasmids were therefore examined using the closely related plasmid R100-1 which also belongs to plasmid incompatibility group FII (ref. 8). As can be seen in Table 2, both the pSC102 and mini pSC102 plasmids are incompatible with R100-1. In 15-h primary transformant colonies, segregation of R100-1 from the R6-5 mini-plasmids is approximately 86% in the case of pSC102 (that is, only 14% of the cells carried both plasmids), and 81% in the case of mini pSC102. As has been observed previously, in incompatibility reactions involving pairs of plasmids derived from a single replicon and hence specifying identical replication-incompatibility systems, segregation of mini R6-5 plasmids and R100-1 was symmetrical, that is, no particular component was preferentially lost²⁰.

In contrast to this result are those obtained from incompatibility reactions involving pBR322 recombinant molecules containing either the *Sal*I fragment 2 or the *Pst*I fragment 6 of pSC102. Table 2 shows that incompatibility reactions involving these plasmids (1) are more severe than mini R6-5 : R100-1 reactions (that is there is usually greater than 98% segregation of the two plasmids during growth of the primary clone); (2) produce a highly asymmetric segregation pattern such that greater than 98% of the cells in the mature primary colony carry one particular member of the original pair of incompatible plasmids; and (3), in the case of the *Sal*I-2 plasmid, frequently result in preferential loss of the pBR322 hybrid plasmid that is known to replicate solely by means of its pBR322 replication functions.

Table 1 Replication systems used by hybrid plasmids

Plasmid	Ratio antibiotic resistant/total <i>polA</i> _{ts} bacteria		Plasmid copy number in wild type
	After growth at 32 °C	After growth at 42 °C	
pBR322 (Ap ^r)	60/60	0/60	27, 38, 33
pSC102 (Km ^r)	56/59	54/59	3, 3, 3
pKT081 (<i>Sal</i> I-2; Ap ^r)	52/52	0/52	25, 22, 25
pKT039 (<i>Pst</i> I-4; Tc ^r)	57/57	0/61	ND
pKT043 (<i>Pst</i> I-6; Tc ^r)	55/55	0/57	29, 29, 28
pKT071 (<i>Pst</i> I-1+4+6 = mini pSC102; Km ^r)	54/58	55/62	4, 4, 3

Replication systems used by hybrid plasmids. The second and third columns show the ability of hybrid plasmids to replicate in bacteria deficient in DNA polymerase I (expressed as ratio antibiotic-resistant/total bacteria). Plasmids were introduced by transformation³² into bacteria of strain KT446 (a *ColE1*-negative, heat-cured derivative of TS214, ref. 33) which produces a temperature-sensitive DNA polymerase I. Purified clones containing the indicated plasmids were inoculated into L broth³⁴ containing an appropriate antibiotic, and incubated at 32 °C overnight. The cultures were subsequently diluted 10⁻¹-fold in L broth that did not contain an antibiotic, and the diluted cell suspensions each divided into two parts: one part was incubated at 32 °C and the other at 42 °C overnight. Each culture was then diluted and plated on nutrient agar at 32 °C for single colonies. These were subsequently picked to nutrient agar plates with and without appropriate antibiotics to test for the presence of the plasmids initially present. It can be seen that pSC102 and the pSC102 mini-plasmid pKT071 are not completely stable at 32 °C in KT446; this slight instability is observed in all strains thus far examined, including *polA*⁺ strains. Nevertheless, the fact that these plasmids do not become more unstable at 42 °C in KT446 indicates that they do not require for replication high cellular levels of DNA polymerase I. In contrast, the *ColE1*-like plasmid pBR322 and hybrid plasmids composed of pBR322 plus *Pst*I and *Sal*I restriction fragments of the pSC102 plasmid are stable when their KT446 host bacteria are incubated at 32 °C, but very unstable at 42 °C, which indicates that pBR322 and the pBR322 hybrid plasmids require high cellular levels of DNA polymerase I for their replication. Cellular levels (copy numbers) of hybrid plasmids in *polA*⁺ bacteria were determined as follows. Single colonies of *E. coli* K-12 CR34 containing the indicated plasmids were picked to tubes of L-broth containing appropriate antibiotics and these were incubated at 37 °C overnight. 0.05-ml aliquots of overnight cultures were used to inoculate 10-ml volumes of M9 minimal glucose medium supplemented with casamino acids (0.5%), vitamin B₁₂ (4 µg ml⁻¹) and thymidine (5 µg ml⁻¹) in 50 ml Erlenmeyer flasks which were incubated in a reciprocal action shake water bath maintained at 37 °C. When the optical density reached an *A*₅₅₀ value of 0.5, methyl-tritiated thymidine (10 µCi ml⁻¹) was added and incubation continued for one hour. Cultures were chilled to 0 °C and the bacteria collected by sedimentation for 5 min at 8,000g in the Sorvall SS34 rotor. Bacteria were washed 3 × with equal volumes of cold fresh culture medium by repeated resuspension and sedimentation, and were finally resuspended in 2 ml of sucrose solution (25% in 0.05 M Tris-HCl, pH 8.0). They were then lysed by the sequential addition of 50 µl of lysozyme (5 mg ml⁻¹), 100 µl of EDTA, pH 8.0 (0.25 M), and 50 µl of a solution of Sarcosyl (20%). Lysis was allowed to proceed at 0 °C for 10 min. Suspensions of lysed bacteria were then vortexed on a rotary mixer for 15 s at top speed to shear chromosomal DNA. 1-ml aliquots were mixed with 7 ml of TE buffer (10 mM Tris, pH 8.0, 1 mM EDTA), 8 g CsCl, and 1 ml ethidium bromide (5 mg ml⁻¹), and the solutions subjected to equilibrium centrifugation in the ultracentrifuge (Beckman 50 Ti rotor), 40 h, 40,000 r.p.m. at 20 °C. After centrifugation, tubes were pierced through the bottom with a needle and the gradients allowed to drip into microtitre plates. Approximately 40 fractions were collected from each gradient and 50-µl portions of each fraction were taken for determination of cold trichloroacetic acid (TCA)-precipitable radioactivity. The proportion of the total TCA-precipitable radioactivity that was found at the position of the covalently closed circular plasmid DNA was used to calculate the plasmid copy number. The values are expressed as plasmid copies per chromosome equivalent and were calculated assuming a molecular weight of 2.5 × 10⁶ for the *E. coli* chromosome³⁵. The individual values obtained from 3 separate experiments are given.

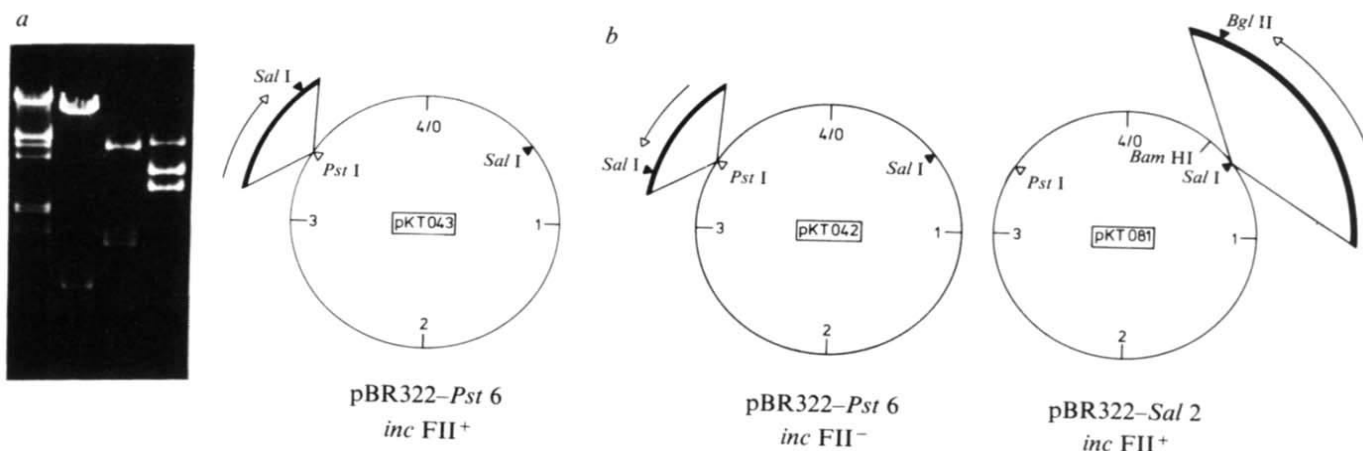


Fig. 4 Structure of *PstI*-6 and *SalI*-2 hybrid plasmids. *a*, Restriction enzyme cleavage analysis of *PstI*-6 and *SalI*-2 hybrid plasmids. Circular DNAs of the *PstI*-6 plasmids pKT042 (*incFII*⁻) and pKT043 (*incFII*⁺) were cleaved with *SalI* restriction endonuclease, whereas that of the *SalI*-2 plasmid pKT081 (*incFII*⁺) was cleaved with *Bam*HI and *Bgl*II. *SalI* and *Bam*HI cut once in the vector molecule; *SalI* and *Bgl*II cut at unique asymmetric locations in the cloned R6-5 fragments. The orientation of the cloned fragments within the vector molecule may therefore be deduced. From left to right: λ DNA cut with *Hind*III and *Eco*RI; *SalI*-2 *inc*⁺; *PstI*-6 *inc*⁺; *PstI*-6 *inc*⁻ (in addition to the two main bands two partial products can be seen in this track). *b*, Structure of the *PstI*-6 and *SalI*-2 hybrid plasmids. The structure and kilobase coordinates of the pBR322 plasmid were taken from Bolivar *et al.*¹⁴ The arrows indicate the direction of the proximal IS1 (IS1b) in the R6-5 plasmid (Fig. 1).

Models for plasmid incompatibility

The disturbance of stable plasmid inheritance observed when two incompatible plasmids come together in a single bacterial clone could result from inhibition of plasmid replication or from interference with the normal distribution of plasmid molecules to daughter cells at cell division (that is, from a disturbance of the plasmid partition process). Models based on mechanisms for the regulation of plasmid replication²¹⁻²³ and on plasmid partition systems^{24,25}, have been proposed to explain the phenomenon of incompatibility. Two models based on the first mechanism have been proposed by Jacob, Brenner and Cuzin (replicon model²¹) and Pritchard, Barth and Collins (inhibitor dilution model^{22,23}). The former hypothesis proposed that specific cellular attachment sites for plasmid replicons are responsible for regulating initiation of DNA replication and for distributing progeny molecules to daughter cells at the time of cell division. Incompatibility was explained by assuming

that the number of attachment sites available is limited and that, whereas compatible replicons utilise different sites, incompatible replicons compete for attachment to the same sites. The latter hypothesis proposed that control of initiation of DNA replication is effected by a trans-dominant, freely diffusible inhibitor substance which limits the frequency of plasmid replication events, and that incompatibility is the result of mutual inhibition of two related plasmids which produce cross-reacting inhibitors. The two models make specific predictions concerning the replication properties of fused (joint) replicons and mutant replicons with altered copy number control, and these predictions have recently been tested. The replication and incompatibility properties of a fused replicon, the ColE1-pSC101 plasmid, that was constructed *in vitro*^{20,26}, and some replication mutants of the plasmid R1 that were isolated by the group of Nordström^{27,28}, are consistent with the predictions of the inhibitor dilution model but inconsistent with those of the replicon model. Thus, the

Table 2 Incompatibility reactions of R6-5 derivative plasmids

Plasmids in primary transformant <i>a</i> <i>b</i>	Total	With <i>a</i>	Cells examined With <i>b</i>	With <i>a</i> + <i>b</i>	% Segregation	Preferential loss
pSC102 (Km ^r); pBR322 (Ap ^r Tc ^r)	65	61	65	61	0	0
R100-1 (Tc ^r); pSC102 (Km ^r)	58	22	28	8	86	—
R100-1 (Tc ^r); mini pSC102 (Km ^r)	57	18	18	11	81	—
pSC102 (Km ^r); <i>PstI</i> -4 (Tc ^r)	61	55	61	55	0	0
pSC102 (Km ^r); <i>PstI</i> -6 (Tc ^r)	57	0	57	0	> 98	+, of <i>a</i> (11/12)
pSC102 (Km ^r); <i>SalI</i> -2 (Ap ^r)	62	59	0	0	> 98	+, of <i>b</i> (15/23)

Incompatibility reactions of R6-5 derivative plasmids. Incompatibility reactions were analysed by following the inheritance of two plasmids brought together in a single bacterial clone by transformation³². In general, incompatibility reactions were so severe that extensive segregation of incompatible plasmids occurred during growth of primary colonies on the selection plates (see also ref. 20). For this reason, it was essential to examine the distribution of plasmids in cells of primary colonies, rather than after purification of transformant clones. Bacteria of strain *E. coli* K-12 CR34 containing plasmids in column *a* were transformed with plasmids of column *b* and plated on nutrient agar (Difco antibiotic medium no. 3) containing pairs of antibiotics that selected transformant cells carrying both plasmids. Well-isolated transformant colonies were resuspended in 1 ml of L broth and the suspension was diluted and plated for single colonies on nutrient agar. Colonies that developed on these nonselective plates were transferred by tooth pick to two nutrient agar plates, one of which contained an antibiotic that could identify continued presence of plasmid *a* and the other of which could identify continued presence of plasmid *b*. Ordinarily, the incompatibility reactions in three or more of each type of transformant clone were analysed and the results of typical reactions are shown. However, in those cases of severe incompatibility where asymmetric segregation of the plasmids was observed to occur, a larger number of reactions was analysed. The partner most often preferentially lost is indicated in the last column, as is the proportion of reactions in which it was lost. The frequency 11/12 indicated for the pSC102/*PstI*-6 reactions means that all cells examined from 11 transformants carried only *PstI*-6, whereas all cells examined from one transformant carried only pSC102. It should be noted that growth of transformant bacteria in medium selective for one or other of the plasmids always resulted in displacement of the nonselected partner. Most hybrid plasmids are described in Fig. 2. R100-1 is a large antibiotic resistance plasmid of the FII incompatibility group and shares extensive sequence homology with R6-5 (ref. 7).

majority of experimental evidence currently available suggests that the mechanism involved in most incompatibility reactions is that of plasmid replication control of the type envisaged in the model of Pritchard *et al.*

A second class of model that has been proposed by Novick^{24,25} postulates a cellular apparatus (for example, membrane sites) for the accurate partitioning of replicons at cell division. This model suggests that the cellular pool of plasmid molecules is randomised before partitioning at cell division and, as a consequence, gross disproportions of distinct plasmids belonging to the same incompatibility group (or partition specificity) will be generated after cell division. Such disproportions cannot be corrected and will tend to be amplified during subsequent divisions, because the partition apparatus cannot distinguish different plasmids of a single incompatibility type. One crucial aspect of Novick's model is that incompatibility and replication control can be independent plasmid functions. To date, there is little published experimental evidence that unambiguously supports or refutes this aspect of the model. In order to determine whether a mechanism other than replication control is involved in incompatibility, it is necessary to be able to observe an incompatibility reaction in circumstances in which replication control cannot function.

DNA fragments that contain an FII incompatibility determinant of R6-5 (pSC102 *SalI* fragment 2 and pSC102 *PstI* fragment 6) have been cloned using the pBR322 cloning vector. Replication and control of replication of the hybrid molecules have been shown to be accomplished by only the pBR322 systems; like pBR322, but unlike the R6-5 parent, the *incFII* hybrid plasmids are present in high copy numbers in host bacteria and cannot replicate in bacteria deficient in DNA polymerase I. Furthermore, they are known to lack DNA sequences essential for R6-5 replication (for example, the origin of replication). The pBR322 plasmid is entirely compatible with, and its replication and copy regulation are not influenced by, the R6-5 plasmid and its pSC102 derivative. If copy control mechanisms only were responsible for incompatibility reactions, and *incFII* is therefore a copy control gene, then the high copy number pBR322 hybrid plasmid carrying *incFII* should destabilise low copy number R6-5 and derivative plasmids, as was observed with the ColE1-pSC101 joint replicon²⁰, but should not itself be destabilised in such reactions. The expectation, therefore, is that a strong asymmetric loss of the pSC102 plasmid should be observed when a pBR322-*incFII* hybrid plasmid is introduced into the same cell. Indeed, this was observed to be the case in a majority of incompatibility reactions between pSC102 and pBR322 *incFII* plasmids. Nevertheless, in a minority of such reactions, especially those involving the *SalI*-2 hybrid plasmid, a strong asymmetric loss of the hybrid plasmid was observed.

One explanation of this result could be that the incompatibility protein coded by the *PstI*-6 and *SalI*-2 fragments binds to a DNA sequence present in these fragments and in some manner hinders or prevents the movement of a replication enzyme complex through the sequence. In this case, pBR322-*incFII* plasmids should either be unstable or at least be low copy number replicons. This was found not to be the case (Table 1). Another possible explanation, that the R6-5 DNA replication control region had been joined to part of the pBR322 control region in the hybrid plasmids, thus forming a fused control region having properties of both plasmids, seems unlikely, because the two *incFII* hybrid plasmids have their R6-5 DNA sequences inserted in opposite orientations at locations in the pBR322 vector which are physically distinct from one another and from the pBR322 replication origin (and presumably replication functions, see Fig. 4). Loss of pBR322-*inc* hybrid plasmids from bacteria concurrently carrying an R6-5 or mini R6-5 plasmid would, therefore, apparently not result from the activity of an R6-5 replication control mechanism.

Incompatibility is an operational term for interactions between two plasmids which prevent their stable co-inheritance. Such interactions may be complex and involve more than one

cellular mechanism. The precise nature and intensity of the interactions between two specific plasmids will govern the particular features (severity, asymmetry) of the observed incompatibility reaction. While some incompatibility reactions may result from the activity of one particular mechanism, others may result from two or more. In the case of pSC102/pBR322 *incFII* incompatibility, a copy control mechanism may well be responsible for the asymmetric loss of pSC102, whereas another mechanism, probably that of plasmid partition, may be responsible for the asymmetric loss of the hybrid plasmid. The intriguing possibility therefore arises that pBR322 *incFII* hybrid plasmids replicate by means of pBR322 replication functions but partition by means of R6-5 partition structures. If this should prove to be the case, then *incFII* designates two different functions or one function involved in two different mechanisms. If the function present on the *incFII* hybrid plasmids responsible for rapid displacement of a co-existing pSC102 plasmid is the pSC102 copy control element, these high copy number hybrid plasmids are likely to direct the synthesis of high cellular levels of this element and therefore should be of considerable value for its isolation and characterisation.

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Note added in proof: By mutual agreement with E. Ohtsubo, we have adopted a new map coordinate system that places the zero position at the terminus of IS1b that is adjacent to the R determinant unit. The new system of coordinates enables direct comparison of map positions related R plasmids in the R1, R6 and R100 series. According to this system, R6-5 *EcoRI* fragment 2 has coordinate 87.5 and 100.8 kilobases (ref. 10) and the DNA sequence carrying *incFII* has coordinates 96.7 kilobases (*PstI* site) and 97.7 kilobases (*SalI* site).

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letters to nature

Deflection of polarised radiation: relative phase delay technique

THE weak equivalence principle states that the world line of a freely falling test particle is independent of its structure and composition. Until recently^{1,2}, experiments designed to detect variations in the geodesic motion of test particles of differing internal properties have been confined to particles of non-zero rest mass³⁻⁵. The experiment discussed here applies the same question to photons by asking whether oppositely polarised photons fall at the same rate.

It is expected that orthogonally polarised photons would be deflected equally by the gravitational field of a non-rotating mass. When rotation is introduced, the angular momentum of the deflecting source couples to the photon spin through the action of the gravitational field. This results in separate trajectories for orthogonal polarisations. In the low frequency limit, that is $M/\lambda \ll 1$, the angular splitting between polarisations, δ , is proportional⁶ to a/λ where M and a are the mass and angular momentum respectively of the deflecting source and λ is the wavelength of the incident radiation. In the high frequency limit, $M/\lambda \gg 1$ and $\delta \propto a\lambda$ (ref. 7). For a solar experiment with $\lambda = 13$ cm and an impact parameter $= 5.5R_{\odot}$, $\delta \lesssim 10^{-14}$ m arc s, a deflection too small to be detected. In our experiment, however, we can set upper limits to the magnitude of this effect. Any other effects that would produce differential deflection of orthogonally polarised radiation components are subject to similar upper limits.

The most sensitive technique to emerge from earlier studies¹ involves searching for a change in polarisation in a deflected beam. If one polarisation mode is deflected more than its orthogonal state, then it will suffer a greater phase delay than the orthogonal mode. The phase delay is a result of the gravitational bending and is approximately 10^5 wavelengths (for 13-cm microwaves) at the solar limb. If any two orthogonal polarisation states suffer differential bending to within 1 p.p.m. of the total deflection, then significant phase differences between the two will be introduced.

Deflection of orthogonal circular polarisations by differing amounts would manifest itself as a rotation of the plane of polarisation. In a single frequency experiment this effect cannot be separated from Faraday rotation in the solar corona, and upper limits can only be set at those solar elongations at which no Faraday rotation is observed. An upper limit of 2.1×10^{-3} arc s was set¹ using the Pioneer 6 occultation data⁸.

If the beam is split into orthogonal linear polarisations, then the final polarisation would, in general, be elliptical. We utilised this technique by searching for a development of ellipticity in the linearly polarised carrier waves from the spacecraft, Helios 1 and Helios 2. The Helios carrier waves are transmitted with the plane of polarisation perpendicular to the ecliptic. Helios 1 was occulted by the Sun in April 1975 and August 1975, and Helios 2 in May 1976. The Helios experiments have the advantages of higher gain in the transmitted signals and lower noise from contamination of the antenna sidelobes due to the Sun (which was near minimum activity in 1975).

Symmetry considerations indicate that any splitting in which orthogonal linear polarisations are deflected by different amounts must involve modes aligned parallel and perpendicular to the radial direction. (In the field of a rapidly rotating object,

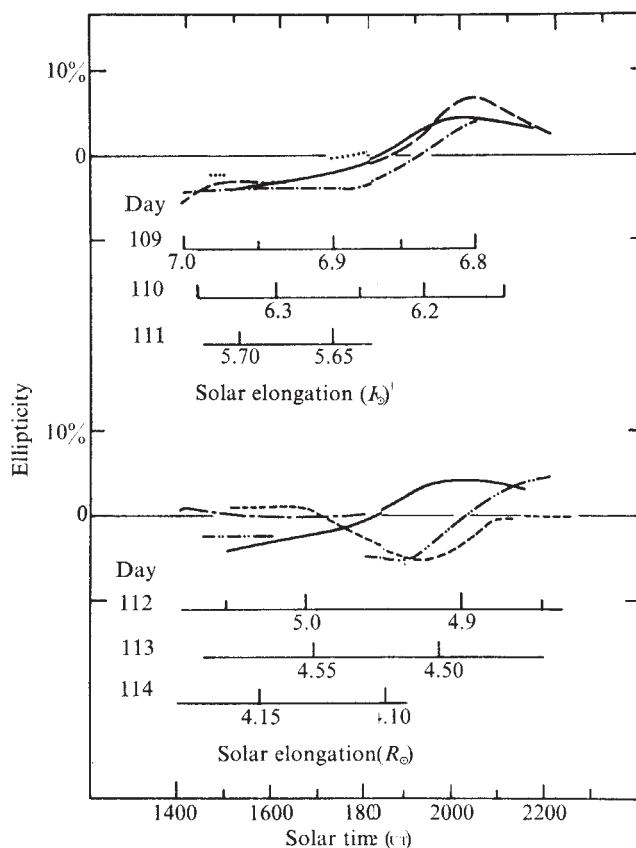


Fig. 1 Ellipticity scans obtained during the April 1975 Helios 1 occultation. The system ellipticity measured on day 96, and reproduced on days 97 and 100 appears as a solid line. The scans for the observing days appear as follows: 109 (—), 110 (---), 111 (....), 112 (---), 113 (---), and 114 (---).

however, this symmetry is broken, and various splittings into differing modes, in different directions are calculable.) In searching for this splitting, Faraday rotation plays an important role because the polarisation of the transmitted signal is aligned perpendicular to the radial direction. After $\pm 45^\circ$ of Faraday rotation the signal becomes an even mixture of these modes at which point the maximum gravitational phase shift could occur. Stelzried, *et al.*⁹ have calculated typical values of the Faraday rotation in the corona of the 'quiet' Sun, and from this we can see that the signal should reach $\pm 45^\circ$ of Faraday rotation at closest approach, within the impact parameter of $4R_{\odot}$ to $7R_{\odot}$. The observed Faraday rotation is usually the result of the net accumulation through several partially cancelling sectors in the magnetic field emanating from the Sun. During the April 1975 occultation Volland *et al.*⁹ observed that the Faraday rotation varied from about 20° to -45° between $7R_{\odot}$ and $4R_{\odot}$. For elongations $\leq 4R_{\odot}$ the magnitude of the Faraday rotation grows rapidly as the elongation decreases.

The Helios 1 carrier wave was monitored for a development of ellipticity on days (of the year) 109–114 inclusive during the April 1975 occultation, and days 244–246 during the August

1975 occultation. The observations were conducted with the NASA/JPL 64-metre antenna of the Deep Space Network at Goldstone, California (DS3-14). A closed loop polarimeter discussed by Stelzried¹⁰ automatically tracked the polarisation of the signal, and monitored the signal for ellipticity. The resulting integrated ellipticity scans are shown in Figs 1 and 2. As the solar elongation decreased the system noise increased due to the interference of the Sun through antenna sidelobes. The ellipticity scans have been averaged with an effective integration constant of about several hundred seconds of observed time and the random errors are comparable to the widths of the curves in the figures. The main limitation seems to be due to the system polarisation which has ellipticity components. Several per cent of systematic ellipticity errors may stem from errors in zeroing of the ellipticity. The system ellipticity was measured by carrying out observations on days 96, 97, 100 and 251 when the angle between Helios 1 and the Sun was large ($\sim 20R_{\odot}$). The instrumental ellipticity was found to depend on hour angle, and to be reproducible from day to day. The resulting curves are shown in Figs 1 and 2. However, when the main beam is near the Sun the curves vary by up to 8% (maximum), and we attribute this to interference from the Sun through sidelobes having different instrumental ellipticity, and possibly to heating of the various parts of the telescope structure. The instrumental effects seem to be the main limitation of this technique.

The data obtained during the April 1975 occultation cover the elongation range $7-41R_{\odot}$. In this range (Fig. 1) Faraday rotation produced optimal conditions for the gravitational phase shift. The observing periods were spaced at frequent intervals with respect to the rate of variation of the observed Faraday rotation, thus any gravitational phase shift should have been detectable during at least one of the periods. The observed ellipticity does not deviate from the system ellipticity by more than 10% (in power), indicating an upper limit to the phase shift between orthogonal components of ~ 0.2 cm. As the time delay for a wave passing within $4-7R_{\odot}$ is $\sim 2 \times 10^6$ cm, then the time delay and therefore the bending should be identical for both linear polarisations to one part in 10^7 of the total bending (at that elongation).

Data taken during the August 1975 occultation extended our results to a smaller elongation than previously studied. Because of the excellent signal-to-noise ratio it was possible to observe Helios 1 to as close as $2R_{\odot}$ (ref. 9). The circumstances of our observations permitted us to obtain the scan of day 244 covering the range $3.3-3.95R_{\odot}$. During this scan Volland *et al.*⁹ recorded a variation in Faraday rotation from about -220° to -40° .

Fig. 2 Ellipticity scans obtained during the August 1975 Helios 1 occultation. The system ellipticity measured on day 251 appears as a solid line. The scans for the observing days appear as follows: 244 (....), 245 (---), and 246 (—).

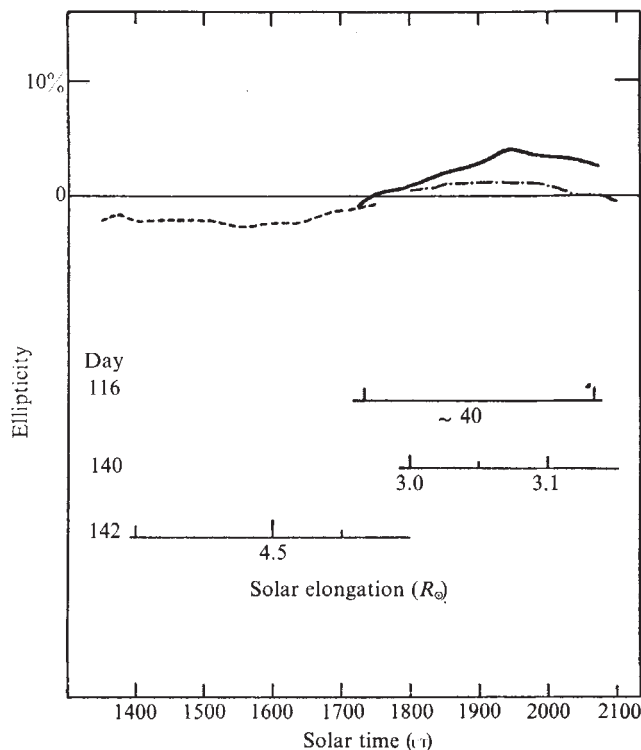
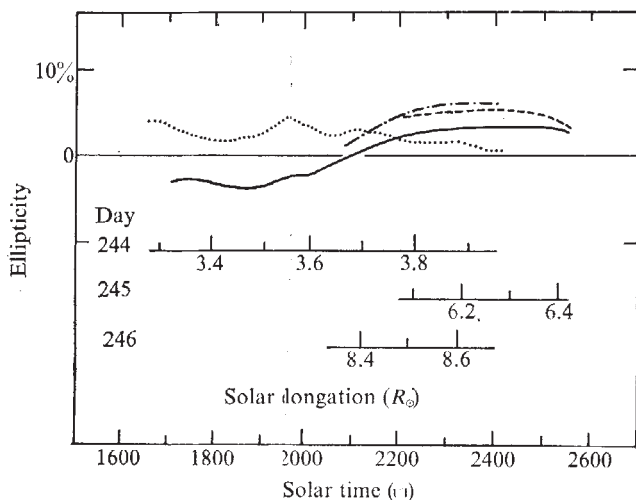


Fig. 3 Ellipticity scans obtained during the May 1976 Helios 2 occultation. The system ellipticity measured on day 116 appears as a solid line. The scans for the observing days appear as follows: 140 (---), 142 (---).

At some time during this period optimal conditions for the gravitational phase shift must have occurred, yet no signal ellipticity exceeding 0.1 was observed, indicating a phase shift between orthogonal components < 0.2 cm. At this elongation, the time delay is $\sim 3 \times 10^6$ cm, and thus the bending of both linear polarisations are identical to a part in 1.5×10^7 of the total bending.

The results from the May 1976 occultation of Helios 2 are shown in Fig. 3. The Faraday rotation on days 140 and 142 was $\approx -40^{\circ}$ (M. K. Bird, personal communication) yet ellipticity in excess of instrumental sensitivity was not detected. This agrees with the upper limit set by the Helios 1 data.

Levy¹¹ carried out a related experiment with the right circularly polarised (RCP) signal of Mariner 4. He looked for a development of left circular polarisation (LCP) when the signal passed within $4.2R_{\odot}$ of the sun. LCP was not detected above the 10% level of total signal power. Differential propagation of orthogonal linear polarisations would produce a mixture of LCP and RCP. These results are further confirmation of a lack of radial splitting between linear polarisations at the level of our upper limits. The phenomenon sought by Levy is based upon a postulated relativistic streaming anisotropy in the solar wind¹², and has the same effect as radial splitting between linear polarisations. The Helios 1 data yield no evidence for this streaming effect, and are consistent with the assumption that the electron streaming velocities equal the proton streaming velocities. We know of no other propagation phenomenon which could mimic or obscure the gravitational effect sought. To an excellent approximation the quasi-longitudinal limit is appropriate for the propagation, and the fundamental plasma modes are circular.

The lack of phase shifts implies that the radial difference in bending between orthogonal linear polarisations is less than 5×10^{-8} m arcs in the overall range, $3.3-7R_{\odot}$. By careful application of the relative phase shift technique we have reduced the upper limits by nearly an order of magnitude below the previously existing values¹. We have extended these results to within $3.3R_{\odot}$ elongation.

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Infrared sources in the vicinity of 2S1728-337

THE X-ray burst source MXB1728-34 (ref. 1), which is believed to be associated with the steady source 2S1728-337 (ref. 2), lies in a heavily obscured portion of the Milky Way. The infrared photometer described previously³, attached to the 1.88-m telescope at Sutherland, was therefore used to search for optically invisible sources at K (2.2 μm) in the region of the error circle of 2S1728-337. The results are presented here: five infrared sources were located.

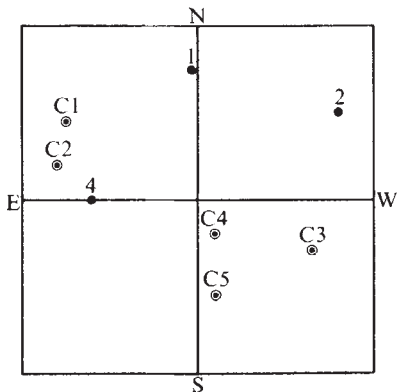


Fig. 1 Map of the 1 arc min² area surrounding 2S1728-337 which was searched in the infrared. The stars 1, 2 and 4 are identified in ref. 2. C 1 to C 5 are the infrared sources. The X-ray error circle has a diameter of 1 arc min and its centre is also the centre of the searched square.

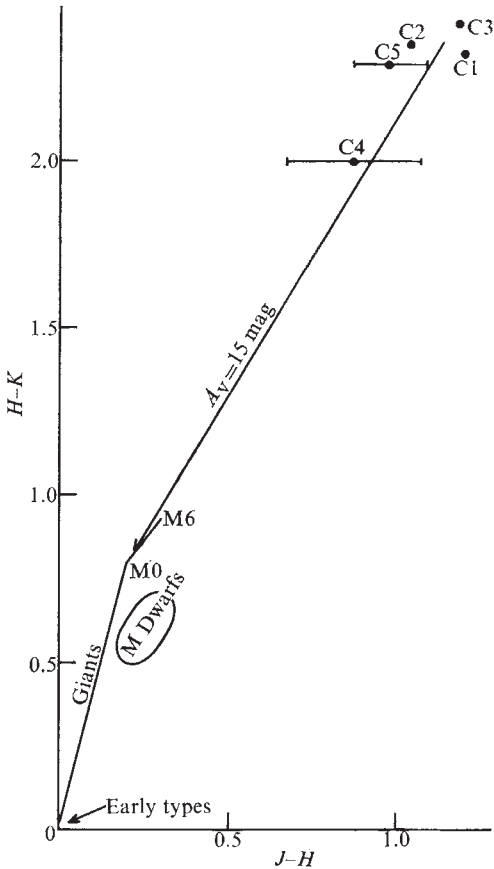


Fig. 2 J-H, H-K diagram showing positions of the five sources and those occupied by normal stars. The effect of removing 15 mag (visual) of reddening is shown.

The search, covering an area 1 arc min² (1'×1'), was made on 23 July 1977. The centre of the search area was located by reference to visible stars. An aperture of 9-arc s diameter was used and the sensitivity of the photometer is such that sources brighter than about K=10 should have been detected.

Five infrared objects were found, and positions were determined to within ±1.5 arc s relative to an arbitrary set of axes. Figure 1 shows the locations of these sources and also those of some visible stars. Each object was then measured to obtain its JHKL magnitudes. These are given in Table 1.

The probability of finding sources of this brightness in a truly comparable region of the sky is unknown (*I*^{II}=354.3°, *b*^{II}=-0.2°). However, previous scans⁴ of the central 1×1 arc min² of the Galaxy yielded only one source brighter than K=8.4. Scans⁵ covering 60 arc min² in areas between *l*^{II}=270° to 338° and *b*^{II}≤1° yielded only nine sources equal to or brighter than K=8; but to a considerable extent these discoveries were expected from OH radio data. Thus the existence of three sources equal to or brighter than K=8.04

Table 1 Infrared sources near X-ray source 2S1728-337					
Source	J	H	K	L	Date (July 1977)
C 1	11.48	9.16	7.96	7.05	27
C 2	11.43	9.08	8.04	7.37	27
C 3	11.31	8.90	7.71	6.91	27
C 4	12.6 ±0.2	10.47	9.60	—	24
C 5	12.48 ±0.11	10.19	9.21	—	24

S.e. 0.05 mag except as stated.

in the 1 arc min² area surrounding 2S1728-337 must be regarded as unexpected. More extensive scanning of neighbouring areas of sky is desirable to determine whether these sources form part of an obscured cluster. Four X-ray bursters have already been identified with the globular clusters NGC6624, NGC1851, NGC6441 and Liller 1 (see ref. 2), so this must be a strong possibility.

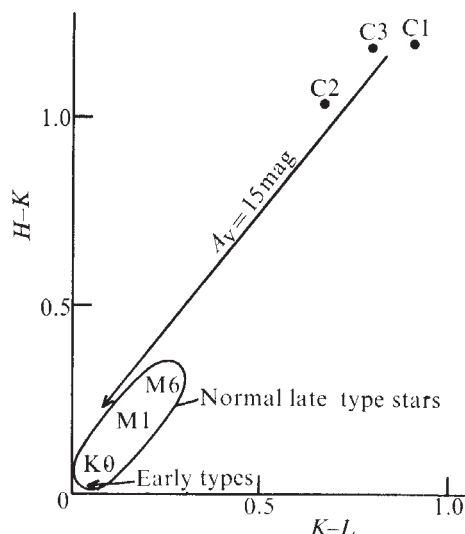


Fig. 3 $H-K$, $K-L$ diagram showing the positions of three sources, and those occupied by normal stars. The effects of removing 15 mag (visual) of reddening is shown.

Figures 2 and 3 show the positions of the infrared sources on the JKH and HKL diagrams. The observed infrared colours are consistent with late-type giant stars reddened by about $A_V=15$ mag. However, knowledge of the reddening law is not sufficiently precise to certainly exclude earlier-type stars reddened by about $A_V=20$ mag. This implies that the dereddened magnitudes of these stars range from $K=6-K=8$ mag. None of them show infrared excesses.

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Oblique refraction of an ionisation front

Gas dynamical effects arising from the absorption of ionising radiation from an O or B group star embedded in interstellar gas have been studied by many authors, and both similarity and numerical solutions have been obtained for the unsteady expansion of the region of almost fully ionised hydrogen gas (HII region) surrounding the star^{1,2}. The boundary between the HII region and the neutral hydrogen comprising the HI region is sharp in comparison with the scale of the motion and is termed an ionisation front. A problem, of interest to both the astronomer and theoretical astrophysicist, concerns the interaction between an ionisation front and a cloud of high

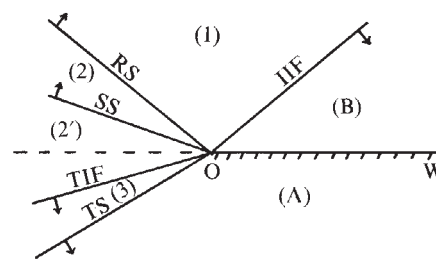


Fig. 1 The oblique refraction of an ionisation front. IIF is incident ionisation front; RS, reflected shock; SS, slip surface; TIF, transmitted ionisation front and TS, transmitted shock.

density molecular gas, as strong observational evidence indicates that dense pockets of molecular gas exist in the vicinity of some O and B stars³. This paper describes an investigation of the interaction between a planar ionisation front and a planar cloud of high density molecular gas. It is found that, in order to determine a unique solution, an extra constraint must be applied which involves the absorption of radiation within the HII region. In two-dimensional flows, however, this condition is limited to flow geometries in which the reflected shock does not interfere with the flow along lines of sight normal to the incident ionisation front. This is analogous to the refraction of shock waves where, beyond a certain angle of incidence, regular refraction will cease and the flow pattern will become irregular^{4,5}.

The normal refraction of an ionisation front was studied by Marsh⁶. Here, a theory of oblique refraction for ionisation fronts is presented. Consider a planar ionisation front propagating into an HI region B, and impinging at an arbitrary angle α on a second HI region A which is much denser (Fig. 1). An ionisation front preceded by a shock is transmitted into region A, and a shock is reflected back towards the illuminating star. The HII region is divided into two parts by a slip surface. Conservation equations can be written for each surface of discontinuity and, since the wave fronts move together along the 'wall' OW without change of shape, we have the following equations connecting the front speeds with the angles:

$$V_1/\sin \alpha = V_2/\sin \phi = V_3/\sin \theta = V_4/\sin \psi \quad (1)$$

where V_1 , V_2 , V_3 and V_4 are speeds of the incident ionisation front, reflected shock, transmitted ionisation front and transmitted shock respectively, and α , ϕ , θ and ψ the corresponding angles the wave fronts make with OW.

An extra condition is required to complete the equations. This comes from considering the absorption of radiation within

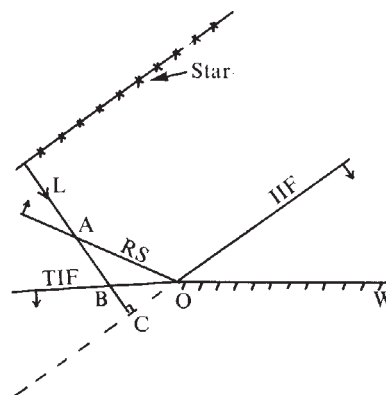


Fig. 2 L is a line of sight from the star normal to the incident ionisation front, meeting the reflected shock and refracted ionisation front at A and B respectively.

the HII region. Measuring the distance r from the incident ionisation front towards the star we have

$$\frac{\partial J}{\partial r} = aJ\rho(1-x)/m \quad (2)$$

and

$$\frac{dx}{dt} = aJ(1-x) - \beta\rho x^2/m \quad (3)$$

where J is the photon flux, a absorption coefficient, ρ density of the gas, m mass of a hydrogen atom, x degree of ionisation, β recombination coefficient and t time. Since the HII region is very nearly fully ionised, that is $x = 1$, equations (2) and (3) reduce to

$$\frac{\partial J}{\partial r} = \beta\rho^2/m^2$$

or

$$J = \frac{\beta}{m^2} \int_0^r \rho^2 dr \quad (4)$$

Referring to Fig. 2 the dashed line shows the position of the ionisation front had no interaction taken place. Since the same number of photons falls on a plane at A normal to L, the number of photons absorbed within the disturbed region AB and that within the undisturbed region AC are the same. Then, assuming the photon absorption at the reflected shock to be negligible, use of equation (4) yields

$$\rho_1^2 \sin(\alpha + \phi) \cos(\alpha - \theta) = \rho_2^2 \sin(\theta + \phi) \quad (5)$$

where the suffixes refer to the regions of flow in Fig. 1. This is the radiation condition required.

The equations must be numerically solved. In general it can be shown that for each set of input data there is only one physically valid solution, and that the present model is applicable only to incident ionisation fronts of the type weak R (supersonic relative to the gases both in front and behind). Also, the density ratio ρ_A/ρ_B must be above a certain value, otherwise more complicated flow patterns will have to be considered.

In deriving the radiation condition we have made a tacit assumption that the line of sight crosses the reflected shock at some finite distance away from the incident ionisation front, that is, that L is not parallel to the reflected shock. This assumption requires a closer look. Rewriting equation (5) in the form

$$(\rho_2/\rho_1)^2 = [1 + \cot(\theta + \phi) \cot(\alpha + \phi)] \sin^2(\alpha + \phi) \quad (6)$$

it can be seen at once that, as $\alpha + \phi \rightarrow \pi/2$, we have $\rho_2 \rightarrow \rho_1$ from above, that is, the reflected shock becomes of zero strength. This particular value of α will be denoted by α^* , and we expect that a change of refraction pattern will take place at this value. For $\rho_2 \leq \rho_1$ the flow will involve a rarefaction wave. However, a simple calculation shows that, if a rarefaction wave occurs, then it will interfere with the radiation condition along L. Hence no regular refractions are possible beyond α^* .

As a direct contrast to shock refraction, an extremely simple expression can be deduced for α^* . As the reflected shock becomes vanishingly weak at α^* , we have

$$V_2 = \phi = V_1 \sin \phi / \sin \alpha^*$$

using equations (1), where c is the isothermal sound speed in the

HII region. But at α^* we have

$$\sin \phi = \cos \alpha^*$$

Hence

$$c \tan \alpha^* = V_1 \quad (7)$$

which is the expression required. For sufficiently fast moving ionisation fronts, therefore, regular refractions are always possible except near grazing incidence.

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Plastic deformation of diamond at room temperature

POLISHED (101) surfaces of type IIB diamonds have been indented with a Knoop indenter at room temperature. The diamonds were thinned from the opposite side by ion-beam bombardment and the resulting deformation observed and analysed using transmission electron microscopy. For the two orientations of indentation examined so far, it has been

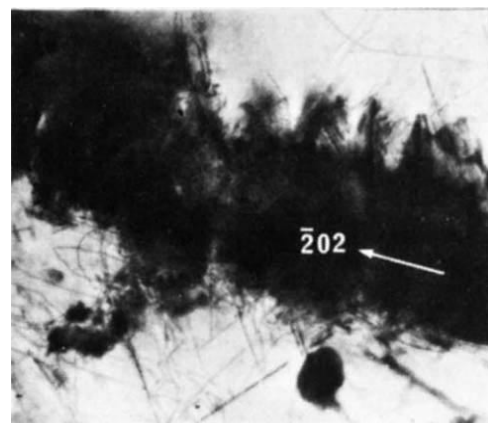


Fig. 1 Transmission electron micrograph of an area near the centre of an indentation in diamond. The long axis of the indenter is orientated close to $[101]$, the diffracting vector is indicated, and the electron beam direction is close to $[323]$. The diamonds were deformed by indenting them at room temperature with a Knoop indenter using light loads ~ 1.47 N (150 g). This type of indenter is a blunt four-sided pyramid, itself made of diamond, which is commonly used for measuring the hardness of brittle materials. It produces indentations which have an elongated lozenge shape. Type IIB diamonds, with polished faces close to the (101) orientation, approximately 100 μ m thick were indented on one face and then thinned from the opposite side by bombardment with a beam of 4 kV argon ions. The indentations were examined under two-beam conditions by transmission electron microscopy at 200 kV. Magnification, $\times 22,500$.

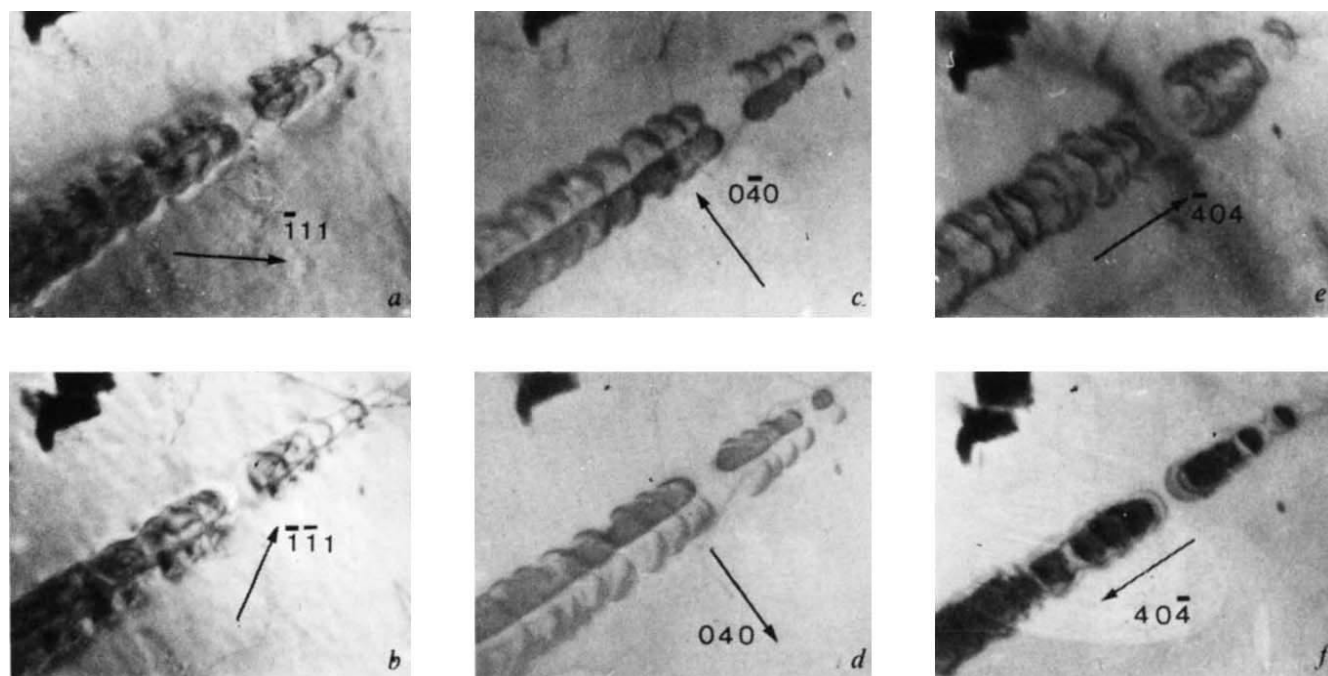


Fig. 2 Six transmission electron micrographs of the area near the tip of an indentation whose long axis is close to $[101]$. The diffracting vectors are marked and the electron beam direction is close to $[101]$. Assuming that the deformation has geometric symmetry about the centre line of the indentation and that similar images arise as a result of similar values of $\mathbf{g} \cdot \mathbf{D}$, the following relations may easily be deduced from these micrographs. From (a) and (b), $-p+q+r = -x-y+z$; from (c) and (d), $-4q = 4y$; and from (e) (or (f)), $-4p+4r = -4x+4z$, where (p, q, r) and (x, y, z) are the components of the displacement vectors $\mathbf{D}$ for the events to the upper and lower sides of the centre line, respectively. Many other relationships for other different diffracting vectors were obtained; all were consistent and led to the values for $\mathbf{D}$ given in the text. The sense of the values $\mathbf{D}$ were obtained using the inside-outside characteristics of the contrast, see for example (e) and (f). Magnification, $\times 16,500$.

found that shear events (probably shear cracks) and dislocations comprise the major part of the deformation. Diamond is usually considered to be a brittle material, easy fracture (cleavage) occurring on $\{111\}$ planes. Although plastic deformation and dislocation generation (also on $\{111\}$ planes) have been demonstrated in diamond at $1,800^\circ\text{C}$ (ref. 1) there has been continuing interest as to whether any plastic flow at all occurs at normal temperatures². Several means of deciding this question have been tried³⁻⁷, but the results are mostly inconclusive. We have indented diamond specimens with a Knoop indenter⁸ and observed the resulting deformation directly using transmission electron microscopy. We report here preliminary results for indentations whose long axes are close to $[101]$ and $[010]$ directions in the (101) surface.

Figure 1 is a transmission electron micrograph of the deformation produced near the centre of an indentation whose long axis is close to $[101]$. The initial dislocation density in these specimens is quite low (see, for example, the background areas in Figs 2, 3 and 4) and thus it is immediately apparent that there has been extensive dislocation generation. However, the intensity of the contrast near the centre of the indentation is so great that no observations could be made on the detailed deformation mechanisms. Analysis, therefore, had to be restricted to regions near the tips of the long axis and to the sides of the indentation.

Figure 2 shows the deformation near the tip of an indentation whose long axis is also close to $[101]$. It can be seen that the deformation has occurred as a discontinuous series of individual events. The events are about 200 nm in lateral extent and are still wholly contained within the thinned specimen which is about 450 nm thick. The extent to which the contrast varies for the different diffracting vectors, $\mathbf{g}$, in Fig. 2 makes it difficult to visualise the geometry of the events from these micrographs alone. How-

ever, tilting experiments in which the specimen was rotated about particular diffracting vectors in turn by up to $\pm 45^\circ$ established that the events are approximately planar and lie close to the (001) planes.

In general, the variation in the contrast of a deformed region as a function of diffracting vector yields information about the displacement field of that region and it was observed that the contrast from these events varied in a particularly systematic way. As a first approximation, we assumed that the displacement field of each event could be characterised by a single displacement vector $\mathbf{D}$. We then used the properties of the contrast, particularly the symmetry and the 'inside-outside' contrast⁹ (see legend to Fig. 2), to investigate values of $\mathbf{g} \cdot \mathbf{D}$ for a large range of diffracting vectors. This procedure yielded consistent values of $\mathbf{D}$; for the events in the upper part of each of the micrographs in Fig. 2, $\mathbf{D}$ is along the $[110]$ direction and for the lower events, it is along the $[1\bar{1}0]$ direction.

As these values of $\mathbf{D}$ lie in the plane of the events, the events are shear events. Because it was impossible to resolve detail in the interior of the events, it was not possible to decide if they were made up of an array of dislocations or were shear cracks. These defects have identical long-range displacement fields on a continuum elasticity model¹⁰ but differ on a short range atomic scale, for example, the amount of rebonding across the slip or shear plane.

To obtain further information about the details of these shear events, a specimen was heated to about $1,000^\circ\text{C}$ for 30 min in the hot stage of the microscope. The details of the contrast from the events were observed to change to that more resembling individual dislocations (compare Fig 3a and b). In several instances dislocation loops were observed to move beyond the previous boundaries of the events. Two obvious examples are shown in Fig 3b. Thus, we think that the shear events are probably shear cracks

which rearrange into dislocation arrays on heating, with the occasional further propagation of dislocation loops. Irrespective of the precise nature of these events, it should be emphasised that they are shear events and therefore constitute plastic deformation of the diamond.

An indentation was made whose long axis was close to $[010]$ and the region near the tip of the long axis is shown in Fig. 4. Many resolvable dislocation loops can be seen along the edges of this indentation and the prominent loops marked A and B in Fig. 4 have been analysed in detail. All these loops lie close to $(1\bar{1}1)$; the loops marked A have Burgers vectors in the direction $[011]$ and those marked B have Burgers vectors in the direction $[10\bar{1}]$. The other dislocation loops lie much closer to the strong contrast region and, although it was apparent that many of them had Burgers vectors in directions other than those determined for loops A and B, no positive identification of their Burgers vectors or slip planes could be made.

For both indentation orientations, cracks with moiré fringes in them (that is, possible tensile cracks) were rare and when they did occur they were confined to regions close to the centre of the indentation.

For the two orientations of indentation examined so far, it can be said that dislocation production is common and occurs near the centre of indentations lying along $\langle 101 \rangle$ directions and along the full length of indentations lying along $\langle 010 \rangle$. Shear events, probably shear cracks, occur near the tips of indentations lying along $\langle 101 \rangle$. The magnitudes of the displacement vectors D of the shear events and of the Burgers vectors of the dislocations have not been determined. We do not know of a method by which the former may be done, and in the latter the dislocations are

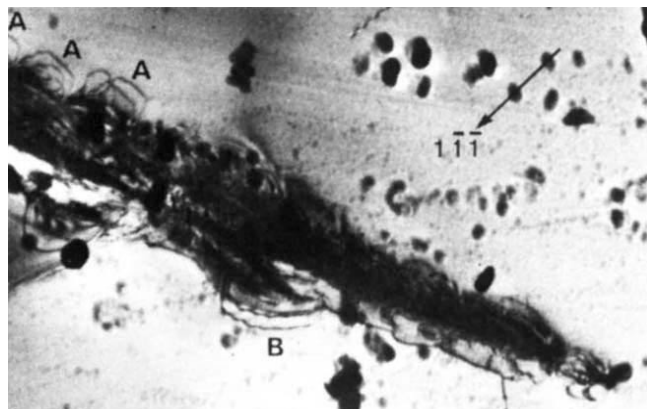


Fig. 4 An area near the tip of an indentation whose long axis is close to $[010]$. The diffracting vector is marked and the electron beam direction is close to $[101]$. Magnification, $\times 21,000$.

too curved and occur too close to the highly deformed region for image matching¹¹ or image doubling experiments¹² to be carried out. However, from the appearance of the contrast, we suggest that the dislocations at A and B in Fig. 4 have the usual $\frac{1}{2}\langle 110 \rangle$ Burgers vectors and the magnitude of each of the displacement vectors D is somewhat greater than this.

The observation of shear events (as distinct from individual dislocations) as a mode of deformation is, in our experience, unique, and their occurrence, close to $\{001\}$ planes rather than $\{111\}$ in diamond is particularly unexpected. The change from this mode of deformation to one of dislocation generation as a function of the orientation of the indentation is also interesting. These problems are being investigated and, with observations on indentations in other orientations, will be reported elsewhere.

These observations show that plastic deformation is the predominant process occurring under the specific conditions of Knoop indentation. On this basis, it seems that the possibility of plastic deformation should always be borne in mind when considering the general mechanical behaviour of diamond.

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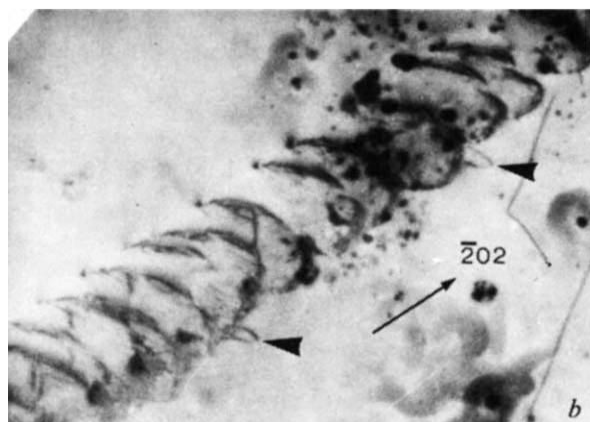
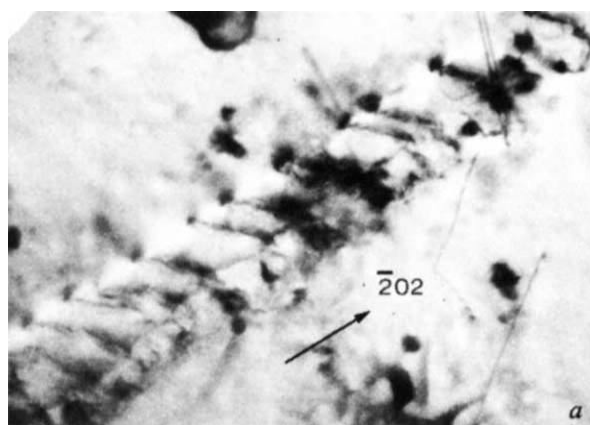


Fig. 3 An area near the tip of an indentation whose long axis is close to $[101]$ before heating (a) and after heating for 30 min at about $1,000^\circ\text{C}$ (b). The diffracting vector is marked and the beam direction is close to $[323]$. Magnification, $\times 34,000$.

Pioneer agriculture explosion and CO₂ levels in the atmosphere

THERE has recently been considerable concern regarding the increasing consumption of fossil fuels. For example, it has been computed that up to 1950, 60×10^9 tonnes of carbon in the form of CO₂ have been discharged into the atmosphere, due to industrial processes such as cement-making and the combustion of coal, oil and natural gas^{1,2}. The changing composition of the atmosphere, and in particular its possible effect on world climate, is a matter of great anxiety. Currently, the CO₂ concentrations in the atmosphere are being monitored at several places on the Earth's surface and it is generally agreed that the levels are increasing by about 1 p.p.m. yr⁻¹ (refs 3–6). This paper points out that even bigger changes occurred in the late nineteenth century due to the sudden expansion of agriculture.

During the latter half of the nineteenth century, there was rapid development of agriculture in the new lands of North America, New Zealand, Australia, South Africa and in Eastern Europe. This 'pioneer revolution' had hardly begun in 1850, yet large areas had been cleared and/or ploughed by 1900. The change was almost synchronous all over the world and must have led to the release into the atmosphere of very large quantities of CO₂. This can be illustrated by considering that a standing forest may contain up to 30,000 tonnes of carbon per km² in the standing wood, and a smaller, but still significant, amount in the litter and soil organic matter, much of which is rapidly lost to the atmosphere once the forest is removed. Similarly, virgin grassland soils have very high organic matter contents of up to 5,000 tonnes km⁻², half of which may be rapidly lost as a consequence of cultivation. An order of magnitude calculation shows that the total carbon released into the atmosphere by man's agricultural practices might have been of comparable magnitude to that released by the consumption of fossil fuel⁷. Although historical records exist on the development of agriculture, details such as the chemical composition of the virgin soil are not known accurately. Here we try to determine independently how much CO₂ was added to the atmosphere from the biosphere, and over what periods.

Because fossil fuels such as oil and coal have ages much greater than the half life of ¹⁴C (5,730 yr) they contain essentially no ¹⁴C. Thus the ¹⁴C/¹²C ratio of the atmosphere provides us with a measure of how much and for how long a ¹⁴CO₂ molecule injected into the atmosphere remains in the gas phase before it is absorbed or exchanged into the ocean. The change in ¹⁴C/¹²C ratio of the atmosphere during the industrial revolution is called the industrial or Suess Effect, and has been determined by measuring the ¹⁴C content of tree rings. The total amount of ¹⁴C-free CO₂ injected into the atmosphere due to the combustion of fossil fuel until 1950 was equal to 9% of the amount already in the atmosphere. On a simple mixing model one would expect the specific activity of the atmosphere to have been reduced by 8%. In fact, it was reduced by only one-quarter of this amount as a result of isotope exchange and sorption into the oceans. Of the CO₂ currently being produced by the combustion of fossil fuel, half is being rapidly absorbed by the ocean, and so for each tonne of carbon released into the atmosphere as CO₂, the carbon inventory of the atmosphere rises by only 0.5 tonnes (refs 1–8). These two facts enable us to compute the fate of the CO₂ injected into the atmosphere by the pioneer agriculturalists.

Standing trees and soil organic matter contain 'modern' ¹⁴C/¹²C ratios (organic matter 80 yr old is depleted in ¹⁴C by only 1%). However, because photosynthesis fractionates strongly against ¹³C, trees, soil organic matter, peat, coal and petroleum have ¹³C/¹²C ratios depleted by about 2.5% (usually expressed –25‰) with respect to the international isotope standard PDB—Pee Dee Belemnite. On the other hand, the CO₂ in the atmosphere is only depleted by 0.7% (or –7‰ PDB). Thus, if we can determine the ¹³C/¹²C ratio of the atmosphere

in the past, we could determine how much CO₂ has been released due to the clearing of forests, draining of peat land and the ploughing of virgin grassland.

One approach would be to measure the ¹³C/¹²C ratio of the wood of tree rings and this has been tried by several workers^{9,10}, not to study the 'pioneer agriculture effect' but to obtain an independent measure of the industrial effect. Unfortunately this attempt was not very fruitful because at the time it was not appreciated what factors other than the isotopic composition of the atmosphere controlled the isotope composition of the carbon in the wood laid down by trees. It is only recently that a serious attempt has been made to study what factors control the fractionation of carbon isotopes by trees. This has been undertaken to obtain past climate information from the isotope ratios of the hydrogen, oxygen and carbon isotope ratios in the various constituents of wood by workers in the embryonic science of isotope dendroclimatology (see, for example, ref. 11). Briefly, the ¹³C/¹²C ratio of a plant constituent such as cellulose reflects the ¹³C/¹²C ratio of the atmosphere, but there is a temperature coefficient¹² of 0.02‰ per °C. This is small enough to be of little consequence in this investigation. A serious problem to be avoided is to ensure that the trees are, in fact, photosynthesising in air representative of the atmosphere, and not in air contaminated by cities or industrial operations¹³. An even more serious problem arises because the atmosphere in closed canopy forests is seriously contaminated isotopically by CO₂ respired from the roots of the trees¹⁴. This CO₂ is made from sucrose translocated into the roots from the leaves, and is isotopically very depleted (–18‰) in ¹³C with respect to the free atmosphere. (The factors which control the fractionation of carbon isotopes in trees and how they can be circumvented are reviewed in ref. 11).

To avoid these problems, wood from a Bristlecone Pine (*Pinus longaeva*) tree was used (University of Arizona Tree Ring Lab number TRL67-3). This tree grew on the lower forest border in the White Mountains of California at an altitude of 3,000 m. Bristlecone pine trees grow as isolated trees exposed to the free atmosphere, and lay down wood for only six weeks at the height of summer each year. (For a more detailed study of the carbon isotopes in Bristlecone Pine see ref. 15). The wood

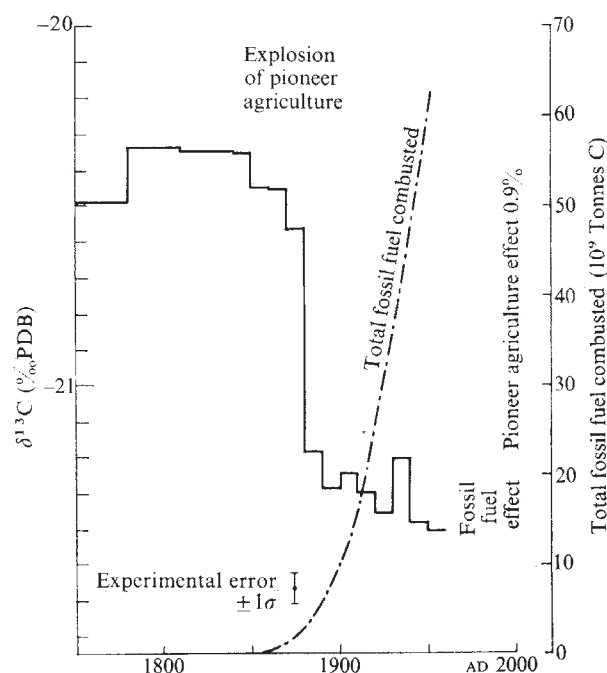


Fig. 1 Plot of $\delta^{13}\text{C}$ values of the cellulose across a section of Bristlecone Pine. Note the sharp change between 1860 and 1890 which occurred before the combustion of fossil fuel had contributed significant quantities of CO₂ to the atmosphere.

was carefully cut into samples which had grown in the years of interest. The samples were ground, extracted with solvent, and cellulose prepared using a scaled down version of the chlorite method of Jayme and Wise as described by Green¹⁶. They were then combusted to CO₂ and measured in a micromass 602C mass spectrometer to a precision of 0.04‰ (1σ). The results are presented in Fig. 1. As can be seen the ¹³C/¹²C ratio changes dramatically from 1870 onwards. The change is vastly in excess of any possible climatically induced changes¹². There seems to be no explanation other than that large quantities of isotopically light CO₂ were liberated into the atmosphere during the period 1860 to 1890. That is before the fossil fuel effect really made a significant contribution (see fossil fuel curve in Fig. 1 and ref. 1).

The data presented in Fig. 1 suggest that the pioneer agricultural effect depleted the δ¹³C value of the atmosphere by 0.9‰. This effect would have been four times this, or 3.6‰, if we corrected for the reduction due to the exchange and absorption into the oceans. Such a change would have been caused by the addition of 110 × 10⁹ tonnes of carbon to an atmosphere which had a δ¹³C value of -6‰ and already contained 550 × 10⁹ tonnes of carbon. Thus the pioneer agricultural effect in the brief period between 1860 and 1890 contributed one and a half times the amount of CO₂ produced by all the fossil fuels burnt up to 1950 (60 × 10⁹ tonnes carbon). The results presented in Fig. 1 suggest that the pre-1850 atmosphere had a δ¹³C value of -6‰ with respect to PDB and concentration of about 270 p.p.m. CO₂, rather than the 300 p.p.m. usually assumed¹⁸.

These data also suggest that as a result of the pioneer agricultural effect, the CO₂ concentration of the Earth's atmosphere suddenly changed over a few decades in the late nineteenth century by about 10% (1/2 of (110/550) × 100). This effect was reinforced and increased during the twentieth century by the growing use of fossil fuels, so that currently the CO₂ levels in the atmosphere have been raised to 330 p.p.m., that is, 22% above the pre-1850 levels. What effect might this have had on the climate of our planet? On many climatic models an increase of 10% in the CO₂ levels in the atmosphere would be expected to raise the world temperature by several tenths of a °C (see refs 19, 20). Until the middle of the nineteenth century it is generally accepted that the Earth was in the cold period known as the Little Ice Age. Although there are shorter term climatic oscillations with a period of decades, it is widely believed that the general level of temperatures during the Little Ice Age was perhaps 0.5 °C lower than, say, the first half of the twentieth century¹⁷. It is suggested that the 10% increase in the CO₂ levels of the atmosphere, due to the pioneer agricultural effect which was later reinforced in the twentieth century by the fossil fuel effect, provided a mechanism whereby the world's temperatures were raised by 0.5 °C in the late nineteenth and early twentieth centuries, and the Earth emerged from the Little Ice Age.

The evidence presented here illustrates that forests and the soil organic matter reservoir represent a very large and potentially controllable pool. Such agricultural practices as growing forests in marginal soils of low initial organic matter content, as is currently being undertaken, for example, on the New Zealand pumice soils, remove very large amounts of carbon from the atmosphere, not only in the form of wood but also in the increased inventory of soil organic matter. The irrigation of desert soils which generally have a low organic matter content will also lead to an increase of the world inventory of soil organic matter. The practice of increasing the intensity of agriculture on existing farm land by the use of irrigation and chemical fertilisers can also lead to an increase in the soil organic matter reservoir. Clearly, some agricultural practices could be an effective and economical way of removing carbon from the atmosphere and should be taken into account in any long term energy policy, if the projected increase in the CO₂ levels due to the consumption of fossil fuels should begin to affect our climate adversely.

These results have important implications for agricultural historians. It seems that there was a period not longer than three decades in the latter part of the nineteenth century when there was a sudden expansion of agriculture much greater than any period before or since. This may have been permitted by the development of railroads, which opened up land not previously accessible. The pioneer agricultural explosion seems to have occurred more or less simultaneously in very different parts of the world—North America, Eastern Europe, New Zealand, Australia and South Africa. The pioneer agricultural explosion which began in the 1870s would be the first and perhaps the most significant of mankind's assaults on our environment at the global level.

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γ-Carboxyglutamic acid in fossil bones and its significance for amino acid dating

AMINO ACID racemisation reactions are promising tools for dating fossil bones¹. However, a major obstacle to their application on a routine basis by geologists, anthropologists, archaeologists and others, is the unresolved question of the effect of *in situ* leaching on apparent racemisation rates^{2,3}. Evidence is presented here that γ-carboxyglutamic acid (Gla), an amino acid recently discovered in fresh bone matrix^{4,5}, is preserved in fossil bone and is a potential quantitative index of leaching for resolving the current debate.

Laboratory simulation studies have indicated that leaching of bone organic matrix can substantially alter the observed stereoisomeric D/L ratios of constituent amino acids and thereby generate misleading apparent ages^{2,3}. Paradoxically, estimated ages of fossil samples based on measured racemisation rates (D/L ratios) are in close agreement with independently determined radiocarbon dates^{6,7}. This concordance in ages for samples of presumably different leaching histories has been interpreted as evidence that leaching has no substantial effect on racemisation rates in the natural environment^{6,7}. However, a definitive test in fossils for a leaching effect depends on the availability of a

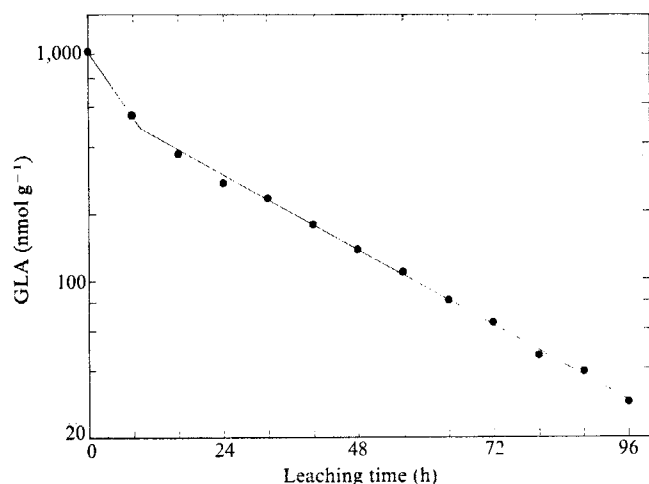


Fig. 1 γ -Carboxyglutamic acid (Gla) concentration in modern bovine bone as a function of leaching time. Bovine cortical bone was obtained from the central section of the tibia of freshly slaughtered cows. The bone was cleaned to remove connective tissue and marrow, thoroughly washed, and ground frozen to a particle size that passed through a 250- μ m sieve. Approximately 100 mg of this modern bone powder was sealed under nitrogen in each of 12 glass culture tubes with 2 ml of ultra-pure water. Continuous leaching was accomplished by maintaining the tubes at 160 °C in a forced-draft oven and by changing the water every 2 h. Samples of the leached bone were withdrawn at 8-h intervals over the total 96-h period for amino acid analysis, including Gla determinations. As Gla is acid-labile and converts to glutamic acid during standard acid hydrolysis, the 2 mg samples used for Gla analyses were subjected to alkaline hydrolysis with 4 M NaOH at 110 °C for 22 h (ref. 14). A measured amount of norleucine was added as an internal standard. Excess cold (0 °C) 12 M HCl was then added to neutralise the hydrolysates and precipitate Na^+ as NaCl. Supernatants were collected after centrifugation, evaporated to dryness in a vacuum centrifuge, and stored in the freezer. Before injection into the analyser, dried samples were dissolved in the first elution buffer, 0.2 M sodium citrate (pH 2.68). A Durrum D-500 amino acid analyser was used at the pmol ($\sim 10^{-10}$ g) level of sensitivity. Total Gla concentration (nmol g^{-1}) was calculated using: (1) a ninhydrin colour yield for Gla of 48% of that for glutamic acid (determined by acid hydrolysis of both synthetic Gla and the Gla-containing bovine prothrombin fragment 1), and (2) the decrease in norleucine to measure the recovery of Gla in sample processing.

an essentially constant rate of Gla disappearance. Although Gla is apparently not a constituent amino acid of collagen¹⁰, it is interesting that the early period of accelerated Gla loss (0–8 h) coincides with the time required for the bulk of the collagen to be removed (K.K., unpublished).

Evidence arguing against a significant contribution of thermal degradation to the progressive Gla decrease includes: (1) the Gla concentration in a sample of modern bovine bone (containing $\sim 8\%$ indigenous water) was unaltered by heating at 160 °C for 24 h in the absence of external water; and (2) glutamic acid, one of the most thermally stable amino acids^{11,12}, disappeared at approximately the same rate as Gla from the leached samples.

Based on the promising inverse correlation between Gla concentration and leaching intensity in modern bone under laboratory conditions (Fig. 1), four fossil bones encompassing a range of probable leaching histories were analysed. Gla was found in measurable amounts in all fossil samples (Table 1). Concentrations of Gla vary by an order of magnitude among the four samples (range: 1,103–101 nmol g^{-1}) and exhibit a trend which is consistent with the results obtained by experimental leaching, that is Gla concentration decreases with increased leaching intensity (Fig. 2). No correlation of Gla content with age of fossil was observed.

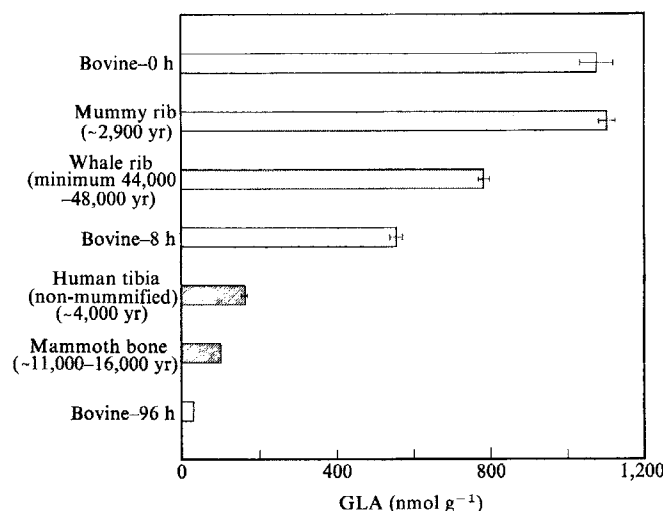


Fig. 2 γ -Carboxyglutamic acid (Gla) concentration in four fossil bone samples. Shaded bars represent the means of duplicate analyses of single fossil samples. Reference values from three artificially leached bovine samples (Fig. 1) are included as open bars. Standard deviations are indicated by lines.

quantitative measure of the *in situ* leaching incurred by the specific fossil sample being dated.

The initial impetus for evaluating Gla concentration as a bone leaching index was: first, the usefulness of amino acid concentration for measuring the intensity of dissolution and leaching of fossil planktonic foraminifera⁸; second, the observation that amino acids from acidic, non-collagenous proteins are leached from modern bovine bone in laboratory simulation experiments at a slow, constant rate (K.K., unpublished); and third, the discovery of Gla in similar acidic, non-collagenous proteins isolated from chicken and bovine bone^{4,5}. In addition it was found that Gla has a very limited distribution in nature and, specifically, has not been detected in microorganisms^{9,10}. Thus, the indigenous Gla concentration in a fossil bone is unlikely to be affected by adventitious organic matter from the environment.

Results from a laboratory leaching experiment with modern bovine bone (Fig. 1) suggest that the absolute concentration of Gla in bone (nmol g^{-1} bone) varies inversely with leaching intensity. The Gla concentration was decreased from an initial value of 1,076 nmol g^{-1} to 28 nmol g^{-1} by continuous leaching for 96 h with ultra-pure water at 160 °C. Additional samples analysed at intermediate 8 h intervals generated a leaching curve composed of two linear segments. The longer segment (8–96 h) reflects

The highest Gla content (1,103 nmol g^{-1}) was found in an Egyptian mummy rib (2,850 $\pm$ 53 yr BP) (ref. 13) which is the same as that of fresh bovine bone (Table 1). This sample is a case of minimal leaching, as it was dissected from a mummy sufficiently well preserved to allow palaeopathological diagnosis of a skin disease¹³.

The next highest concentration of Gla (780 nmol g^{-1}) occurred in a fossil whale rib sample with an estimated minimum age of 44,000–48,000 yr BP (G. H. Miller, personal communication). This sample was collected from the permafrost layer of a raised marine delta in a fjord at Baffin Island, Canada. Geologic and palaeoclimatic considerations suggest that this sample had been totally protected from leaching in permafrost for most of the time since burial. Alternating conditions of freezing and melting probably occurred seasonally for some unknown portion of the past 8,000–9,000 yr; this would have resulted in some leaching. The observed Gla content of this sample is 28% lower than the concentration in unleached modern bovine bone.

Table 1 γ -Carboxyglutamic acid (Gla) contents of four fossil bone samples

	Mummy rib (PUM-1)	Whale rib	Human tibia- non-mummified (12th Dynasty)	Mammoth bone
Gla concentration* (nmol g ⁻¹)	1,102.9 (22.5)	779.6 (16.4)	165.1 (7.0)	101.3 (3.3)
Ratio of Gla (sample) to Gla (fresh bovine bone)	1.02	0.72	0.15	0.09
Estimated age (yr)	2,850 $\pm$ 53	$\sim$ 44,000–48,000 (minimum age)	$\sim$ 4,000	$\sim$ 11,000–16,000
Site location	Unknown (Egypt)	69° 28'N 67° 33'W (Baffin Island)	29° 27'N 31° 05'E (Egypt)	39° 41'N 102° 31'W (Colorado)
Probable leaching history	None	Limited leaching in permafrost/active layer during past 9,000 yr	Intensive leaching	Intensive leaching

*Mean values and standard deviations (in parentheses) for duplicate analyses of single fossil samples.

Dense cortical bone was separated for analysis from the more porous, cancellous sections of the fossil bone samples. After removing and discarding several millimetres from all exposed surfaces, the bone fragments were cleaned ultrasonically in a series of dilute sodium hexameta-phosphate washes and ultra-pure water rinses, and then air dried. Alkaline hydrolysis and Gla analysis of each 2-mg sample were conducted as described above (Fig. 1). An additional preparative step for fossils involved the filtering of the final diluted sample with a Flath-Lundin syringe (0.22 μ m polycarbonate membrane filter) before injection into the analyser.

The extremely low levels of Gla in the other two fossil samples, an Egyptian non-mummified human tibia (165 nmol g⁻¹) and a mammoth bone (101 nmol g⁻¹), were not unexpected. Both were recovered unprotected from depositional environments conducive to leaching. In addition, low nitrogen contents (relative to modern bovine bone) had previously suggested extensive *in situ* leaching of the organic matrix in both samples (P. E. Hare, personal communication).

The preliminary field data represented by these four fossil samples, when compared with the Gla contents of systematically leached bovine bone samples, strongly suggest that Gla concentration of fossil bones and *in situ* leaching intensity are inversely correlated in the natural environment (Fig. 2). Fossil Gla contents are delimited by the experimental values obtained in bovine bone with maximum and minimum levels of laboratory leaching. Also the fossil samples are clearly segregated into two discrete groups by their Gla values which in turn correlate with low-intensity leaching (high Gla) and high-intensity leaching (low Gla). The difference in Gla content between the two samples in each group is statistically significant and each Gla value is interpreted as a measure of the relative leaching intensity incurred by each sample.

An alternative to the leaching hypothesis is that intrinsic intra- and/or interspecies Gla variations among these samples have generated a fortuitous, non-causal relationship between the degree of leaching and Gla content. Although adequate mammalian baseline data are not presently available to eliminate this possibility unequivocally, the striking 85% difference in Gla content between the two human fossils of similar age (Table 1, Fig. 2) is difficult to explain by any mechanism other than the known difference in leaching histories.

Based on the above preliminary laboratory and field data, Gla concentration seems to be a potentially useful index of *in situ* leaching in fossil bones. The possible role of leaching in the alteration of the D/L ratios used for the amino acid dating of fossil bones can be investigated directly with this new tool.

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Intense progressive shear in Ile de Groix blueschists and compatibility with subduction or obduction

THE Ile de Groix (South Brittany, France) is made up of a series of metapelitic rocks intercalated with metabasic horizons, most of which contain glaucophane^{1,2}. Palaeozoic metamorphism^{2–4} in the basic rocks produced various assemblages, such as glaucophane + lawsonite^{5,6} or omphacite + garnet, characteristic of high pressure and low² to medium¹ temperatures. All these rocks were later slightly or completely recrystallised under greenschist facies conditions^{1,5,6}. The presence of a contrasting metamorphic facies (high temperature/low to medium pressure), on the mainland north of Groix, has led several workers to propose the existence of a paired metamorphic belt² of pre-Hercynian age^{4,7,8}, formed as a result of subduction^{2,7–9}. We present evidence here concerning the style of deformation contemporaneous with the high-pressure metamorphism.

The rocks on Groix contain a variety of structures. Cogné *et al.*⁵ identified flat-lying folds with northerly trending axes and suggested that these formed during a first deformation phase and were further flattened during a second synmetamorphic phase. Two later and relatively minor postmetamorphic phases

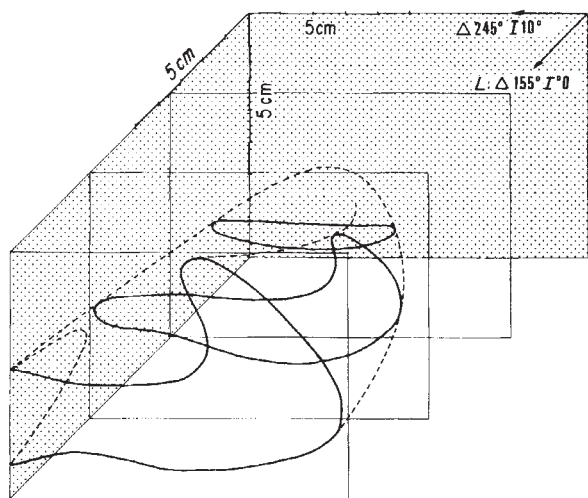


Fig. 1 Isometric projection showing sheathlike form of folded surface of quartzitic layer (Vallon de Kerigant, Ile de Groix). Vertical planes represent serial sections normal to linear structure (L). Traces of folded surface in each section are closed curves (thick solid lines). The form of folded surface (dashed lines) has been obtained by interpolation. Sample is orientated (Δ = declination; I = inclination).

were also described. Boudier and Nicolas¹⁰ proposed an even earlier deformation which 'resulted from laminar motion, the direction of the movement perhaps being indicated by a mineral lineation (glaucofane and lawsonite, trending N 120° E–N 140° E). No folds necessarily need be associated with this deformation'.

Our study leads us to emphasise the following structural features. First, the rocks display a composite planar structure of tectonic and metamorphic origin. This structure is on average nearly horizontal (later complications neglected) and is composed of several grossly parallel layerings and foliations, containing recumbent isoclinal folds. Second, the rocks also display a composite linear structure which has a northerly trend (north-north-west in the north-west of Groix) and is composed of (1) several lineations, including intersection lineations and stretching lineations; (2) elongate pressure shadows around syntectonic garnet porphyroblasts; (3) quartz rods; (4) long axes of boudins formed in competent basic horizons; and (5) hinges of isoclinal recumbent folds.

We have studied these folds in two ways, statistically and individually. Statistically, analyses of axial directions of fold populations show that most axes trend nearly north–south, but a few have east–west or intermediate trends. Individually, most folds are noncylindrical, often highly so, hinges being curved within the axial surfaces. For several hand specimens of folds developed in quartzitic or basic horizons, we have established fold morphologies by cutting serial sections normal to the local linear structure (Fig. 1). Characteristically, the folds have the form of flat elongate sheaths¹¹ lying within the composite planar structure and pointing along the linear structure. Natural outcrops of such sheaths (Fig. 2) are rare, but convincing. The largest sheath so far identified has a nose region about 3 m wide at exposure (Vallon du Lavoir, Quéhelo). In general most sheaths are asymmetric about their axial surfaces, one limb being longer than the other; but variations in the sense of asymmetry, from fold to fold, have not yet been studied systematically. Where rocks are multilayered, the sheath form generally persists through many successive layers, but the amplitude diminishes from a central maximum: thus folds appear to have been generated internally, not by displacements transmitted from neighbouring structures.

Some folds are known to be more noncylindrical and sheath-like than others. In the more extreme examples, the nose region

occupies only a small part of the total volume and the lateral margins are cylindrical and nearly parallel. The proportion of folds exhibiting such a form remains to be established, but two factors make this study difficult. First, good marker horizons (such as the quartzitic layer of Fig. 2) are rare. Second, the nose regions, critical in the identification of sheaths, are infrequently exposed. It is possible that many fold hinges, exposed locally and trending nearly north–south, are in fact the lateral margins of sheaths.

The relatively simple form of some sheaths may be deceptive. In some instances we have observed one sheath superimposed on another. The details of superimposition have yet to be established, but first results suggest that sheaths may have developed successively in time.

From our structural observations on Groix, we draw the following conclusions concerning the state of finite strain and the history of deformation. First, the form and orientation of sheaths suggest that the bulk strain ellipsoid everywhere has a principal axis of extension, X , nearly parallel to the composite linear structure, and a principal axis of shortening, Z , nearly normal to the composite planar structure. The forms of pressure shadows around syntectonic garnets support our interpretation and suggest also that there has been little or no finite extension along the intermediate axis, Y . Other structural evidence, including grain shape fabrics (the rocks are LS-tectonites) also support our interpretation. We have not attempted any quantitative strain analyses, because adequate markers have not been identified.

Second, the presence of folds with differing degrees of noncylindricity suggests either that finite deformation is heterogeneous, or that folds initiated at differing times. There is little correlation of fold noncylindricity with other structural evidence (such as grain shapes) and therefore we favour fold initiation at different times and suggest that differing degrees of noncylindricity represent successive stages of fold development.

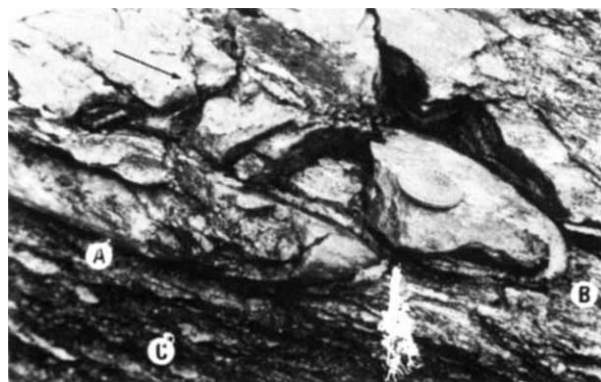


Fig. 2 Photograph of outcropping sheathlike folds of quartzitic layer embedded in micaschist (Vallon de Kerigant, Ile de Groix). A, Long lateral margin of sheath. B, Rounded noses of sheaths. C, Near vertical section through micaschist, showing composite planar structure. Arrow indicates composite linear structure (declination = 150°; inclination = 5°).

If so, noncylindricity may be due to progressive rotation of fold hinges towards X , from initial orientations close to Y (refs 11–16). Reorientation may also have affected boudins, most of which now have long axes nearly parallel to Y .

The existence of superimposed sheaths and of several nearly parallel foliations suggests that the bulk strain history was noncoaxial. This is supported by our observation that pressure shadows around syntectonic garnets are asymmetric. Within some garnets, the presence of sigmoidal inclusion trails, showing apparent rotations far greater than 90°, is a strong indication that deformation was continuously rotational.

The simplest kind of progressive deformation, that is at once continuously noncoaxial and continuously rotational, is a progressive simple shear. We suggest that the structural evidence from Groix is satisfied by a model of progressive deformation with a major component of simple shear (Fig. 3), the shear planes being nearly parallel to the composite planar structure (and the XY plane), the shear direction nearly parallel to the linear structure (and X). Recent work on mylonitic shear zones¹¹, where the finite deformation is known quantitatively and the deformation history is unequivocal, has shown that noncylindrical folds can develop during such a deformation. Noncylindrical folds and associated structures have also been described from the base of a nappe¹⁶, where there is strong evidence for progressive shear. If the major component of deformation on Groix is indeed simple shear, then the total shear strain must be of large magnitude, because many fold hinges are now very nearly parallel to X and individual sheaths are often extremely elongate, more so than those described in shear-zones and nappes^{11,16}.

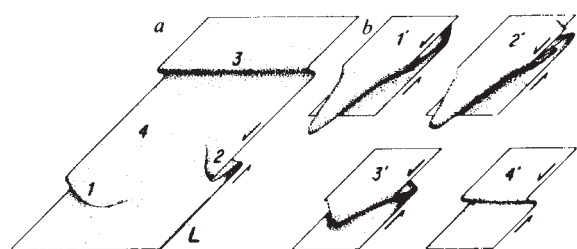


Fig. 3 Hypothetical model of development of sheathlike folds. Overall deformation is progressive, with strong component of simple shear (arrows). At earlier stage of deformation (a), folded surface shows folds at various stages of development (1 = moderately curved hinge; 2 = sheathlike fold; 3 = near cylindrical hinge; 4 = site of future fold nucleation). At later stage of deformation (b), all pre-existing structures have been modified (fold 1 has become sheath 1'; sheath 2 has become refolded within elongate sheath 2'; hinge 3 has become noncylindrical fold 3'; site 4 is now occupied by near cylindrical hinge 4').

The bulk sense of shear on Groix has not yet been determined unequivocally, but may perhaps be deduced from systematic analyses (in progress) of fold asymmetry and of microstructures in and around syntectonic garnets. The significance of glaucophane orientation in the basic rocks of Groix also needs to be studied: the orientation is extremely variable and may not be simply related to a shear direction.

The regional implications of our structural analysis are as follows. First, the model involving progressive shear and large shear strains is compatible with the existence of a subduction zone (with possible obduction) in which rocks were transported to depths sufficient to generate high-pressure metamorphism. Further work on the sense of shear on Groix may provide strong evidence for distinguishing subduction from obduction. Second, the present north-south trend of the linear structure (including most fold hinges) on Groix is strongly oblique to the general trend of the high-temperature metamorphic belt on the mainland. Such an obliquity is said to be characteristic of orogenic zones with belts of shear deformation¹⁵. Unfortunately, there is as yet little evidence to indicate whether or not the trends in Brittany have been modified by late tectonic displacements post-dating plate convergence (Lefort and Segoufin, personal communication). In the absence of such modification, the north-south trend on Groix can be interpreted as a direction of subduction or obduction relative to the mainland. The inclination of the shear-zone during these processes is not known.

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Sampling and observation of oceanic mantle and crust on Gorringer Bank

THE structure and nature of the uppermost oceanic mantle and crust has been, and still is, the subject of a major international research effort¹. Yet the direct field evidence known to us on this subject, whether obtained by drilling in the I.P.O.D. oceanic crust programme² or by submersible surveying in the Famous programme³, has been either very incomplete or limited to the upper part of layer 2. As a result, the problem of the comparison between ophiolites and oceanic crust is still debated¹. We report here the preliminary results of an *in situ* geological survey in which we have sampled and observed what we believe to be an almost complete geological section starting a few kilometres below the Moho discontinuity and ending in the upper part of layer 2 (ref. 4). This section outcrops on the flanks of Gorringer Bank, which is a ridge 200 km long and 60 km wide between Tagus and Horseshoe abyssal plains in the Atlantic ocean, South West of Cape Saint Vincent, Portugal (Fig. 1).

Gorringer Bank lies on the northern boundary of the seismic belt which defines the Africa–Europe plate boundary between the Açores triple junction and Gibraltar. In the area of Gorringer Bank, the Africa plate is presently underthrusting the Europe plate in a south-south-east–north-north-west direction⁵. A very large, 350 mgal, gravimetry free-air anomaly is associated with the Bank, whereas the seismic belt to the south-east is characterised by a -80 mgal negative anomaly^{6,7}. Le Pichon *et al.*⁷ described the structure associated with this seismic belt as 'an incipient trench between two oceanic plates' colliding at a relatively slow rate. Gorringer Bank was assumed by them to be a piece of oceanic crust uplifted at the time of formation of the trench, 5–10 Myr ago. This interpretation led to the implantation of hole 120 of the DV Glomar Challenger which indicated the presence of Barremian (115 Myr) sediments lying directly over oceanic basement and suggested the existence of a significant amount of uplift (several kilometres) during the Miocene^{8–10}. The hypothesis of Gorringer Bank as a piece of oceanic crust uplifted and slightly tilted in Neogene time together with its original

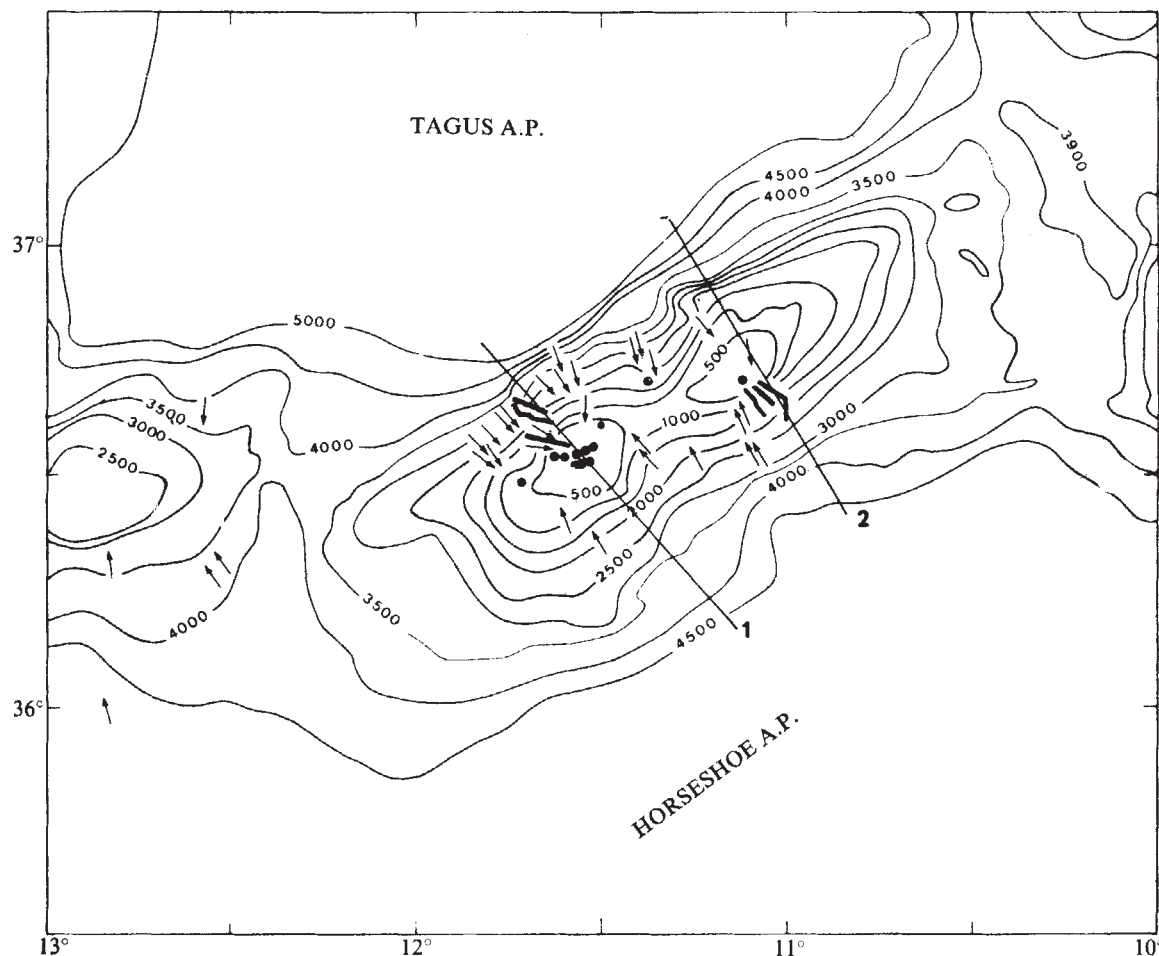


Fig. 1 Location of dives and sample sites on Gorrige Bank. (1) and (2) are bathymetric profiles shown on Figs 3 and 4. Heavy lines, zones surveyed by submersible dives; ●, core; → dredge.

sedimentary cover was widely adopted⁸⁻¹¹. It was further pointed out that the age of the sedimentary cover agreed with the assumed age of opening between Iberia and North America¹².

Since then, however, a large amount of oceanographic work has suggested the importance of the Mesozoic and Palaeogene complex evolution of the Africa-Iberia plate boundary¹³⁻¹⁷ and showed that the transform motion has been prevalent during a great part of its history. It thus seemed reasonable to associate Gorrige Bank with a palaeo-transform fault with which it had many structural and petrological similarities (refs 8-10, 16, 18, J. Cann, personal communication). In this interpretation, Gorrige Bank would be a diapir emplaced at an initial time along the Transform Fault (see ref. 19). A geological field survey of Gorrige Bank's flanks with the submersible Cyana of the Centre National pour l'Exploitation des Océans was made in August 1977 to test the validity of these interpretations.

Gorrige Bank lies in a south-west-north-east direction between the Tagus Abyssal Plain to the north and the Horseshoe Abyssal Plain to the south (Fig. 1). It is 250 km long and 100 km wide. Its southern flank has a thick sedimentary cover whereas the northern flank is steeper with widespread large-scale slumping and consequent outcropping of the acoustic basement. The basement also outcrops near the summit where it pierces the sedimentary cover, forming two massifs which culminate at 25 m water depth, Gettysburg to the south-west and Ormonde to the north-east.

Two *in situ* geological sections have been obtained. The first starts at a water depth of 2,600 m on the northern flank and ends on the summit of Gettysburg. Twenty-two

successful sampling stations were made during four submersible dives along this north-west-south-east section. In addition, orientated samples were obtained on the summit (25-40 m) by skin-diving. The second section starts at a depth of 1,800 m on the southern flank and ends on the edge of Ormonde at 200 m. Twelve sampling stations were made during three dives along this south-east-north-west section (Fig. 1).

On the basis of dredges in the area (Nøratlante, Nestlante and Gibraco cruises of CNEXO, and J. Cann, personal communication), we had assumed that Gettysburg was essentially made of serpentinite. This was confirmed by the first profile (Fig. 2) which demonstrates that the serpentinites form a major part of outcrops and keep a remarkable structural and chemical homogeneity from top to bottom. These are former harzburgites, with a north-south banded structure dipping at 20° to the East. Evidence of high temperature plastic flow is present in most specimens. The orientated samples obtained on the summit by skin-diving show a foliation, due to high temperature plastic flow, with a similar orientation. A 200 m high cliff, made up of micro-litic dolerites, at a depth of 1,250 m, corresponds either to a sill or rather to a thick flow. Dredgings had already shown the existence of a Cainozoic volcanic activity of this type¹⁵. The serpentinites are widely covered by recent mud and by consolidated sediments of Cenozoic and Lower Cretaceous (Barremo-Aptian) age. Barremo-Aptian was also the age obtained by the Glomar Challenger for the sediments lying immediately above the basement.

The slope is also covered with serpentinite blocks and pebbles. Breccias made up of serpentinite blocks in a detrital calcareous matrix outcrop on steep scarps on the slope

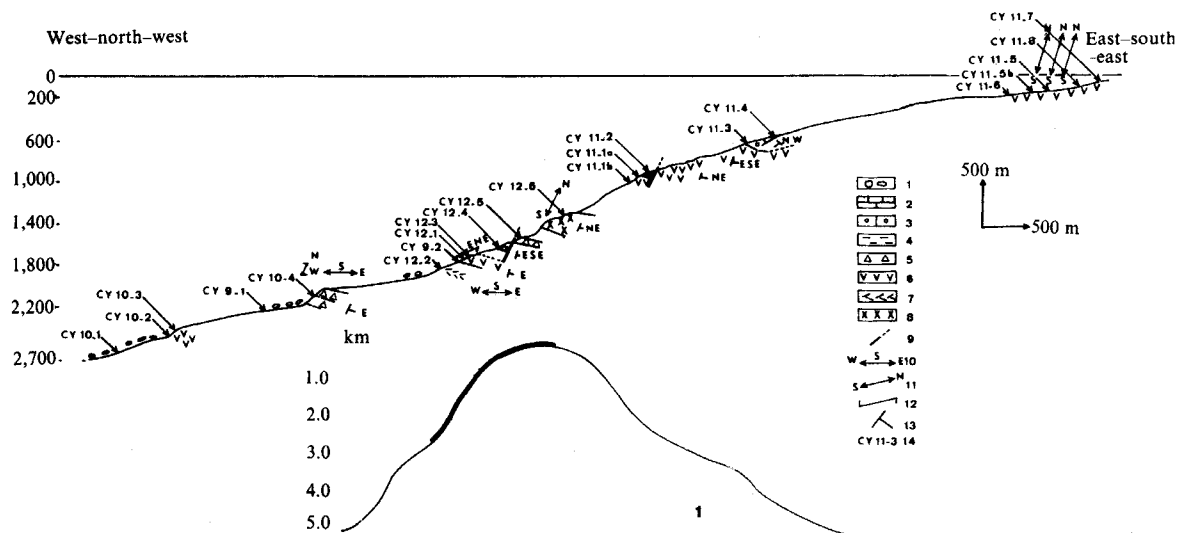


Fig. 2 Synthetic section of the north-west flank of Gettysburg seamount. Dives 8, 9, 10, 11, 12. 1, isolated blocks; 2, limestones; 3, conglomerate; 4, Albo-Aptian marls; 5, serpentinites breccias; 6, serpentinites; 7, microlitic lavas; 8, dolerites; 9, fault; 10, schistosity lineation; 11, fracture trends; 12, horizontal movement; 13, bedding dip; 14, samples collected during dives.

(Fig. 2). We believe that they are not due to tectonic activity of diapiric or faulting type as listric surfaces have not been observed. They are probably the result of slope rubble consolidation. Listric surfaces have been observed in massive serpentinite along nearly vertical fault planes with variable directions.

Thus Gettysburg consists of massive structurally homogeneous serpentinite over a thickness of at least 2.6 km, with no indication of diapiric activity, suggesting that it is a piece of mantle. Because of the frequent presence of gabbros in the dredging made over Ormonde, we had assumed that it might correspond to a piece of oceanic crust, the 20° tilting to the East accounting for the dissymmetry between Ormonde and Gettysburg. This was verified by the three dives made on the southern slope of Ormonde.

Figure 3 shows that between 1,600 and 800 m, the slope consists of a monotonous rise covered with mud, with a few scarps made up of neogene limestones and conglomerates with volcanic pebbles. There is, however, a thick outcrop of volcanic breccias near 1,000 m. Between 800 and 450 m there are high cliffs made up of apparently

fresh gabbros having an irregular structure with finer grain zones alternating with 'pegmatoidal' zones. They have a vague orientation with a 20° dip to the east or north-east. This orientation is apparently tectonic as bands of highly foliated gabbros cut the massive gabbros along the same orientation. Six bands, from one to a few metres thick, have been observed over 60 m of cliffs in gabbro. Towards the top, doleritic dykes become more and more numerous. Their orientation seems to be quite constant: north-south with a 70 to 90° dip to the east (10 measurements). Near 450 m, dykes are so numerous that the formation should be considered to be a dyke complex. The fine grained and greenish intruded rock might correspond to an altered basalt. It is at this level that apparently basaltic pillow-lavas have been observed in the rubble. The section ends near 200 m on the edge of Ormonde plateau in trachytic and phonolitic very fresh explosion breccias. These breccias are most probably related to the Cainozoic acid volcanism¹⁵.

Gorringe Bank is independent of the Iberian continental margin and is situated in an oceanic environment between Tagus and Horseshoe Abyssal Plains (5,000 m depth)^{8,11,14,16}.

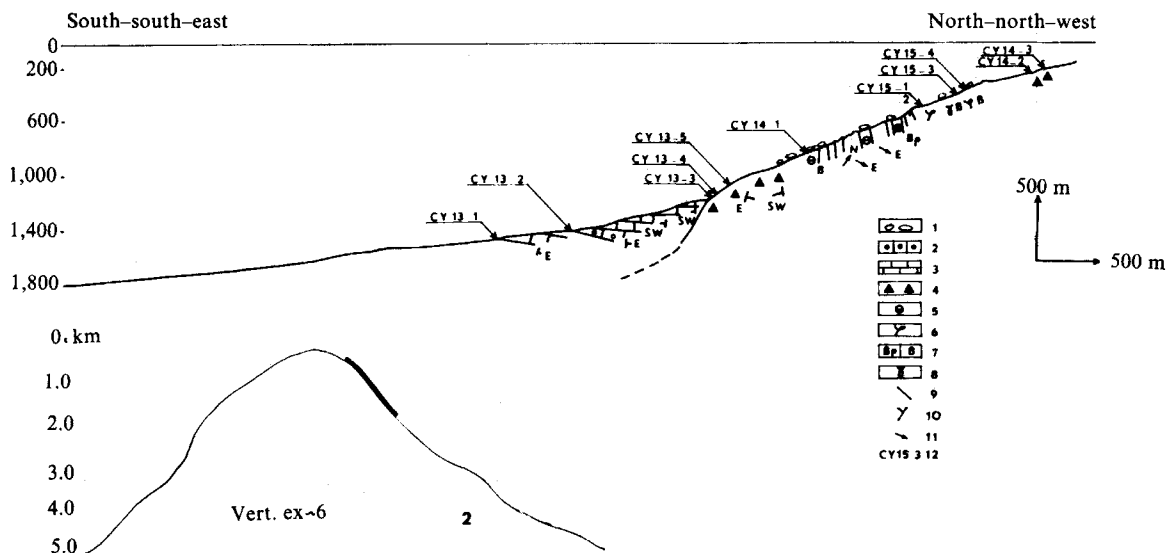


Fig. 3 Synthetic profile of the south-east flank of Ormonde seamount (dives 13, 14, 15). 1, isolated blocks; 2, conglomerate; 3, limestones; 4, volcanic breccia; 5, gabbros; 6, phonolites; 7, basaltic pillow lavas and basalts; 8, metagabbros; 9, diabase dykes; 10, bedding dip; 11, foliation lineation; 12, samples collected during dives.

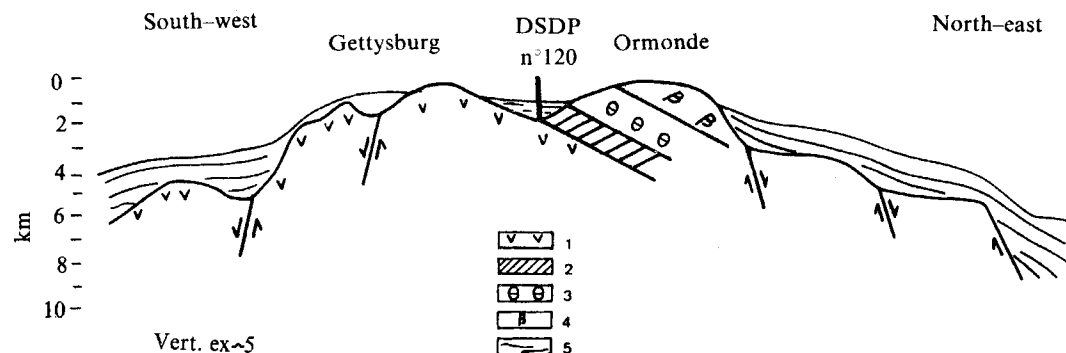


Fig. 4 Interpretative longitudinal section of Gettysburg and Ormonde seamounts. The sequence of the various layers is shown. 1, Serpentinities; 2, intermediate bodies; 3, gabbros; 4, basalts; 5, sedimentary layers.

The preceding descriptions present numerous affinities with an ophiolitic complex in which Gettysburg is at its base (serpentinities with a tectonite structure) and Ormonde at its top (Fig. 4). However, we have not observed the chromite dunites and the gabbroic cumulates expected in the intermediate section which may lie below 800 m in Ormonde. Similarly, we cannot prove that the dykes and the pillow lavas near the edge of Ormonde plateau are basaltic. They might be due to the Cenozoic acid volcanism. In spite of these reservations, we conclude that Gorringer Bank is a piece of oceanic crust and that the interpretation which assimilates an ophiolitic complex to a fragment of oceanic mantle and crust has been justified in the field.

Another conclusion of these observations is that the structure seems not to be disturbed in a major way by faulting tectonics. A recent morphologic survey made before the dives suggests, however, that the bank is cut by north-north-east-south-south-west faults with probably a left-lateral component of motion. We still do not have enough information to date the events after the formation of this fragment of oceanic crust. We simply note that the oldest sediments (Barremo-Aptian) obtained using a submersible have the same age as those obtained by drilling, and apparently lie directly on top of the serpentinities on the west, whereas they lie on top of gabbros and ultrabasics at the drilling site. This suggests, but does not prove conclusively, that the ultrabasics were already outcropping in Barremo-Aptian time. In this case the tilting of Gorringer Bank could be linked to the development of the very large ridge corresponding to J anomaly (110 Myr Aptian)² or with the initiation of spreading at the Jurassic-Cretaceous boundary. The survey has also confirmed the importance of an acid volcanic phase that can be linked with Cenozoic tectonism^{15,17}.

Thus, the Gorringer Bank would be the result of an uplift of about 10 km of the mantle and overlying oceanic crust, due to a slight eastward tilt. We found no evidence of major deformations in the analysis of the structures of serpentinities and sediments. The zones of highly tectonised gabbros correspond to horizontal thrusts within the oceanic crust. They provide direct evidence for the existence in the oceanic crust of an important thrusting in various metamorphic conditions. This hypothesis has been proposed for our recent studies in ophiolites^{21,22}. In this interpretation, the saddle between Gettysburg and Ormonde (Fig. 1) would correspond to the deeper part of the oceanic crust. Alternatively, the general structure might be explained by a downward movement to the east of the fault suggested by the morphology between the two massifs.

From our results it seems that a nearly complete oceanic crust section and 4 to 5 km of underlying upper mantle are outcropping on the flanks of Gorringer Bank.

The conformity between the different basement layers and the lack of evidence of strong tectonic disturbance lead us to reject the hypothesis of a diapiric origin for the Gorringer Bank. Instead, we think that the present configuration of the Bank results from a gentle eastward tilt

of the oceanic basement followed by vertical uplift. Also noteworthy is the fact that the surveyed section appears very similar to the standard section of an ophiolitic complex.

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Date of deglaciation of Mount Elgon

HIGHER East African and Ethiopian mountains are known to have been extensively glaciated during the Quaternary^{1,2}. After Mount Meru in Tanzania, Mount Elgon (4,321 m), on the Kenya/Uganda border, is the highest such mountain to be completely free of glaciation today. We present here ¹⁴C determinations which indicate that deglaciation of Mount Elgon occurred shortly before 11,000 BP.

Mount Elgon is a Miocene volcano surmounted by an 8 km diameter caldera. Despite earlier indications to the contrary¹, we have been able to distinguish only one major glacial stage on the mountain. This is marked, *inter alia*, by prominent termino-lateral moraines within valleys radiating out from the caldera rim (altitude ~3,950–4,250 m). The lower-most moraines reach down to altitudes of ~3,350 m. Valleys on the southern slopes of the mountain are headed by ice-scooped rock basins, at least two of which still contain lakes. Basal organic sediment has been collected at two sites within one of these lake basins (4,150 m; 1° 06' N, 34° 34' E; grid XS 749215) and submitted for ¹⁴C determination. The samples, which are from sediment depths of 385–405 cm and 625–655 cm, respectively, give dates of 11,012 ± 135 BP (SRR-1118) and 10,708 ± 230 BP (SRR-1120).

On Kilimanjaro³, Mount Kenya⁴ and Ruwenzori^{5,6}, several series of major glacial advances have been identified. On the basis of the appearance and altitude of the moraines, the Elgon glaciation is believed to be correlated with the last main glaciations on these mountains (the Mahoma Lake glaciation on Ruwenzori)^{1,5}. Only one ¹⁴C date has previously been published for a major glacial episode in tropical Africa⁷. This date of 14,700 ± 290 BP is from basal organic sediment infilling a kettle-hole lying on a terminal moraine of the Mahoma Lake stage.

The origin of the ice-scooped rock basins on the southern Elgon slopes is not well understood. They may be associated with resistant rock strata. Alternatively, or additionally, they may mark either a period of standstill during general ice retreat or a minor glacial re-advance. In either case, the 11,000 BP date for deglaciation is concordant with that from Mahoma which refers to the date of retreat from maximum ice advance and, therefore, provides evidence to support the theory that glacial episodes are correlated on the different East African mountains. If the ice-scooped rock basins on Elgon originated during a standstill phase or a minor ice advance, then they may correlate with the Omurubaho glaciation on Ruwenzori, marked by moraines at altitudes intermediate between those of the Mahoma Lake glaciation and the present glacier limits^{5,6}.

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Modification of heat resistance in *Drosophila* by selection

WHAT functional component of an animal is most sensitive to heat? We discuss here two reasonable possibilities which have quite different implications. If heat injury is mediated primarily by enzyme inactivation, then natural selection may well have brought a large number of enzymes to the point where a given duration at a high temperature should cause a comparably critical loss in the activity of each. If this is the case, then selection for increased heat resistance would require simultaneous responses at many enzyme loci, as one sensitive enzyme would make the increased stability of the others worthless. In genetic terms, the sensitive enzyme would be epistatic to the rest. On the other hand, if cell or tissue damage is the first effect of excessive heat, then heat resistance is likely to be a quantitative characteristic, with a considerable additive genetic component. Selection for increased thermostability should be possible. In either case, selection for reduced heat resistance should be relatively rapid, as temperature-sensitive mutants would, as a class, probably be frequent enough to dominate the situation.

Although Waddington¹ pioneered the use of indirect selection for response to heat shock, successful selection for resistance has never, to our knowledge, been reported, although we and others² have observed strain differences in heat sensitivity. We have now succeeded in increasing heat resistance within an isofemale line of *Drosophila melanogaster*. Selection for decreased resistance was much more rapid and most of the response seemed to be due to a single allele.

Forty isofemale lines were started from wild inseminated females caught in June and July 1976, in five Iowa locations. These were cultured under standard conditions. Indirect selection was started at once and carried out as follows. From each line or subline, 60 flies were treated. Untreated siblings in the four lines with the greatest (or the four lines with the least) survivorship were allowed to produce 10 sublines each, and the process was repeated. Two transfers were required to produce the necessary numbers, and so selection was carried out about once per month. By the 5th generation, upward selection was proceeding within one isofemale line, and downward within another. Both originated in Chariton, Iowa. Treatment involved the exposure of adults, 10 males and 10 females per vial, to 40 °C for a given period (initially 25 min). The vials contained no food; cotton plugs were moistened and forced into a position well below the surface of a Haake water bath, controlled to within 0.1 °C, into which the vials were immersed. Flies to be treated were etherised and placed in empty vials 1.5–3.5 h before treatment. Figure 1 is a graph of survival, in which 5-min dosage shifts are equated to 0.33 survivorship. The precision of this conversion factor, which is based on actual comparisons, is not critical to the conclusions.

After 10 generations of selection, comparison was made between the upward and downward lines and the unselected lines derived from each of their ancestral isofemale lines: the results are listed in Table 1. The difference in survival between the

Table 1 Survival after 30 min at 40 °C

Stock	Alive	No. dead	Total	Proportion alive
Selected Low	0	2,394	2,394	0
Unselected Low parental	752	1,509	2,261	0.333
Unselected High parental	892	1,478	2,370	0.376
Selected High	1,507	850	2,357	0.639

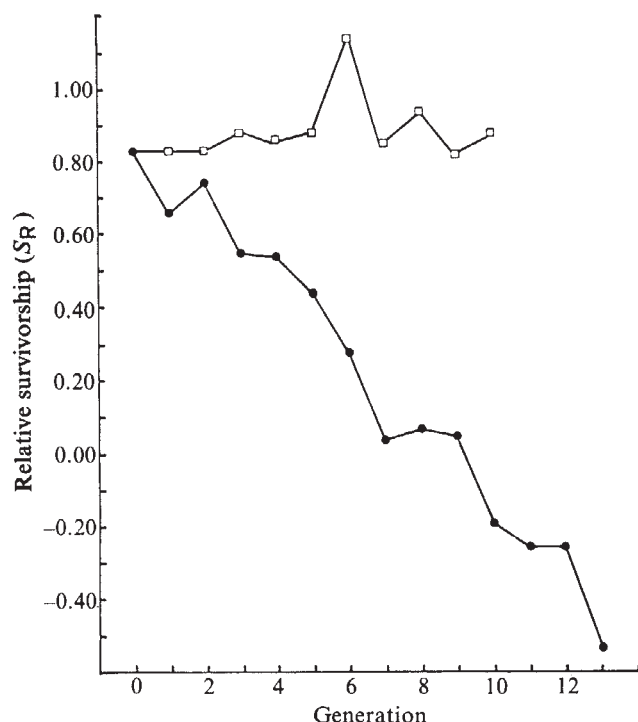


Fig. 1 Response to selection for increased and for decreased resistance to heat shock. Duration, t , was modified as needed during selection. S_R proportion surviving $+ 0.33(t-25/5)$. Thus a more accurate reflection of the response to upward selection is seen in Table 1. $\square$, Selected high; $\bullet$, selected low.

selected high line and its unselected ancestral line is substantial and highly significant: in a sign-test of 119 matched pairs of vials, the division was 103+ and 16- ($P \approx 10^{-16}$). The selection response of the low line is much greater. The two unselected lines themselves did not differ significantly.

The genetic basis of the downward response was analysed in terms of chromosomal contributions by the use of the cross illustrated in Fig. 2. Chromosomal analysis was performed with the aid of a balanced marker stock, Cy/bw^{V1} ; D/Sb . Cy and bw^{V1} are on the second chromosome; D and Sb are on the third³. F_1 males carrying the wild X chromosome and the markers Cy and Sb were backcrossed to wild females, and progeny were treated at 40 °C for 25 min. As the survivorship of the respective marker classes indicates, there is a large difference between flies homozygous for the wild 'low' second chromosome and those heterozygous for Cy . In flies heterozygous for chromosome II, the third chromosome makes a small additional contribution ($\chi^2 = 5.3$; $P < 0.02$). The simplest explanation is that a single locus is the major contributor to the downward response. This interpretation seems the more

Fig. 2 Diagram of crosses and table of responses of each backcross-progeny class to 25 min at 40 °C. Unmarked chromosomes from the marker stock are designated $+^M$.

$\begin{array}{c} \text{X} \\ \text{+} \\ \text{+} \end{array} \times \begin{array}{c} \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \end{array} \begin{array}{c} \text{Cy} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \end{array} \begin{array}{c} \text{D} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \end{array} \begin{array}{c} \text{Sb} \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \end{array}$			
$\begin{array}{c} \text{+} \\ \text{+} \end{array} \times \begin{array}{c} \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \end{array} \begin{array}{c} \text{Cy} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \end{array} \begin{array}{c} \text{D} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \\ \text{+} \end{array} \begin{array}{c} \text{Sb} \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \\ \text{+}^M \end{array}$			
Progeny classes			
$\frac{+}{+} \frac{+}{+}$	$\frac{++}{++}$	$\frac{++}{++}$	$\frac{++}{+^M}$
1	394	395	0.3
$\frac{+}{+} \frac{+}{+}$	$\frac{++}{++}$	$\frac{++}{+Sb}$	$\frac{++}{+^M}$
2	390	392	0.5
$\frac{+}{+} \frac{+}{+}$	$\frac{++}{+Cy}$	$\frac{++}{++}$	$\frac{++}{+^M}$
243	152	395	61.5
$\frac{+}{+} \frac{+}{+}$	$\frac{++}{+Cy}$	$\frac{++}{+Sb}$	$\frac{++}{+^M}$
272	119	391	69.6

likely in view of the apparent recessivity of the effect. Perhaps some 10% of the response is due to additive (polygenic) effects. The upward response was not considered great enough to justify similar analysis at this point.

Further downward selection has since been achieved, and the test temperature has been lowered to 38 °C in order to continue our experiments further.

As all the selection was carried out within isofemale lines, the results confirm the presence of considerable potential genetic variability in isofemale lines of *D. melanogaster*^{4,5} and promise greater response in both directions when more genetic variation is available. It is, of course, possible that a factor stabilising many enzymes at once is under quantitative, polygenic control. Preliminary experiments have so far failed to reveal correlated variation in the stabilities of enzymes.

The upward response to selection for thermostability may thus be taken tentatively as favouring the hypothesis that heat injury to organisms is not mediated by way of enzyme inactivation.

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Mitotic coincidence of chick embryo hepatocytes *in vivo* and the transition probability model of the cell cycle

THE transition probability model of the cell cycle^{1,2} was proposed in order to account for the high variability of intermitotic times always observed in cell populations. According to this model, the cell cycle is divisible into an A state of indeterminate length, and a B phase of fixed length during which the activities of the cell are directed towards division. Cells enter the A state sometime after mitosis. The transition from the A state to the B phase in G_1 is a probabilistic event, and it is the stochastic nature of this transition that generates the observed variation. Although the model provides an adequate explanation of the proliferation kinetics *in vitro*²⁻⁶ and *in vivo*^{1,7,8}, there is direct experimental support, time-lapse cinematography studies of intermitotic times only for those *in vitro*²⁻⁵. Therefore, the applicability of the new model *in vivo* is still controversial⁵. We show here that an original kinetic parameter, the mitotic coincidence of neighbouring cells (M_{co}), can be predicted accurately, on the basis of the new model, using independently measurable variables, for cell populations growing *in vivo*. We present the theoretical basis for the computation of this parameter according to the classical model and according to the new one. Our results in chick embryo hepatocytes, in close agreement with the latter, are direct evidence of the applicability of the transition probability model *in vivo*.

The random transition assumed in the new model originates the high variability of intermitotic times in cell populations as a whole and also between sibling cells. The classical model, in which no random transition is accepted, can explain the overall variability of the population by environmental heterogeneities in the space, or by little differences in cell composition that accrue from successive divisions, but cannot give an appropriate explanation for the high variability between siblings, if cells are able to divide accurately. The sibling pairs variability

is, therefore, a good test to distinguish between the two models. In cultured cells, the time-lapse studies have shown exponential distributions of differences in intermitotic times between sibling cells (β curves)³⁻⁵, in close agreement with the predictions of the new model. For technical reasons, β curves cannot be constructed *in vivo*, but we have found an original way to measure the variability of generation times between siblings, that can be applied to 'solid' cell populations growing *in vivo*.

The mitotic coincidence of neighbouring cells is a kinetic parameter related to the degree of synchrony between sibling cells. When a parental cell divides, the siblings generally remain adjacent. This can be assumed in 'solid' tissues, as the epithelial ones, where the cells being attached by junctional complexes cannot move independently. In mammalian liver parenchyma, junctional complexes occur near the bile canaliculus^{9,10} and they can be also found at the same site in chick embryo hepatocytes (Fig. 1). Thus the degree of synchrony between siblings must affect the proportion of adjacent cells that are in mitosis at the same time, and thus the frequency of mitotic pairs in the histological sections. We can define M_{co} as the probability that any cell adjacent to a given mitotic cell will itself be in mitosis.

Thus,

$$M_{co} = n(1-s)MI(t) + nsSP_{co}(t) \quad (1)$$

where M_{co} is the proportion of mitoses associated in pairs in the tissue section (Fig. 2), n is the average number of cells adjacent to any given cell in the two-dimensional tissue section (determined as the number of nuclei surrounding every nucleus), s is the probability that a cell is the sibling of a given neighbour (determined as one divided by the average number of neighbouring cells in three dimensions), $MI(t)$ is the mitotic index at time t , and $SP_{co}(t)$ is the fraction of sibling pairs that will be in synchronous mitosis after a mitotic block of duration t . The first term in the right hand side of equation (1) is the probability of finding one mitotic cell among the unrelated neighbours of a given reference mitotic cell. The second term is the corresponding probability for the sibling of the reference cell.

The two models differ decisively in their prediction of $SP_{co}(t)$. In both cases, the necessary and sufficient condition for mitotic coincidence of a sibling pair is that any difference in their time of progression through the cell cycle should be less than the time of mitotic block (t). $SP_{co}(t)$ is thus derived as the fraction

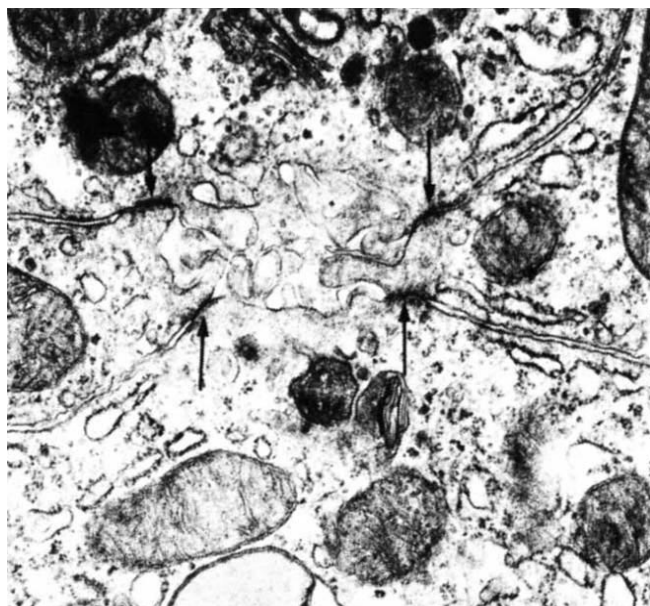


Fig. 1 Electron micrograph of liver parenchyma of 15-d chick embryo. Bile canaliculus formed by four hepatocytes attached by junctional complexes (arrows). $\times 25,000$.

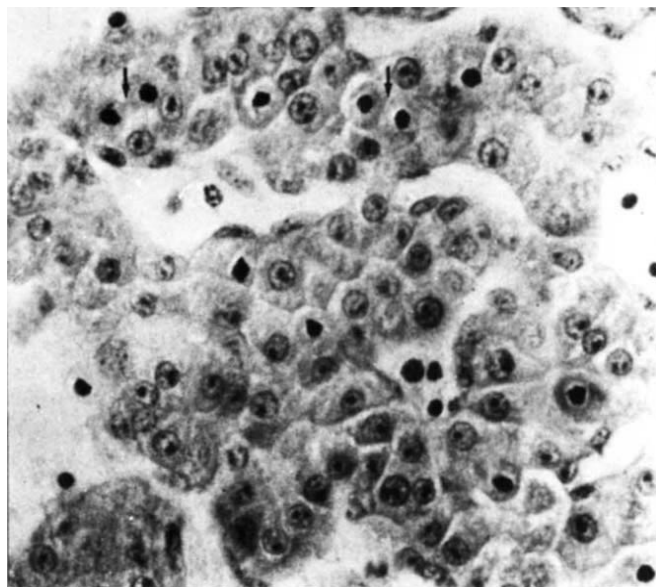


Fig. 2 Blocked mitoses in the liver parenchyma of 10-d chick embryo. Mitotic coincidence (M_{co}) is the proportion of mitoses associated in pairs (arrows), (mitoses in pairs/mitoses) in the tissue section.

of siblings with a delay in progression less than t . According to the transition probability model, the fraction (β) with a cell cycle delay equal to, or greater than, a given time is an exponential function of time. So, at time t after the beginning of the block, $\beta(t)$ pairs will be non-coincident and $1-\beta(t)$ pairs will be coincident, that is

$$SP_{co} = 1 - \beta(t) = 1 - \exp(-kt) \quad (2)$$

where k is the first-order rate constant for the transition.

In the classical model, assuming a normal distribution of differences in intermitotic times between siblings, with an average difference of 0 and a coefficient of variation CV :

$$SP_{co} = 2 \int_0^t 1/(2\pi)^{1/2} \exp(-1/z^2) dz \quad (3)$$

and

$$z = t/CV.GT \quad (4)$$

where GT is the average generation time, t is as above, and z is t expressed as the standard score (the product $CV.GT$ is the standard deviation). Expression (3) is the percentage area of the normal curve occupied by the sibling pairs with intermitotic time difference less than t .

We have investigated the proliferation kinetics of hepatocytes in chick embryo liver by introducing colchicine or vinblastine mitotic blocks at various times from day 5 to day 20 of development. The average generation time (GT), the corresponding proliferation rate constant (PK) and the mitotic time (MT) were determined by standard methods¹¹⁻¹³ (Table 1). (A full account of these experiments will appear elsewhere. (M_{co} was determined after $t = 6.0$ h (14-d embryo) or after $t = 4.5$ h (10-d embryo). From equations (1) to (4), the theoretical value of M_{co} was then computed for the two models. The stereological parameters n and s were measured from sections and three-dimensional reconstructions of the same preparations used for MI and M_{co} determinations. $SP_{co}(t)$ was computed according to the transition probability model by deriving k from PK and the B phase time (T_B) according to Smith and Martin². T_B is not given by the mitotic block method, and was therefore approximated by taking the minimum GT that we have observed for these cells, that is $GT = 16$ h for the 5-d embryo. This approximation is a valid one since T_B is approximately constant for a particular cell type and variations in GT

Table 1 Kinetic parameters of chick embryo hepatocytes from the 5th to the 20th day of development

Age (d)	GT (h)	PK (h ⁻¹)	MT (min)
5	16.3	0.0426	
6	24.3	0.0286	
7	31.4	0.0221	
8	38.2	0.0181	
10	52.5	0.0132	50
14	55.7	0.0124	50
17	64.9	0.0107	
20	72.2	0.0096	

The coefficient of variation for all values (GT, PK and MT) in the Table is about 10%. 60–70-g eggs (New Hampshire × Leghorn) were incubated at 37–38 °C and the mitotic index (MI) of the liver parenchyma was measured from the 5th to the 20th day in 5 µm histological sections. 10 µg of vinblastine per egg was injected in the air chamber of 10-d and 14-d embryos and the average generation time (GT), the respective proliferation rate constant (PK) and the mitotic time (MT) were determined for these two stages according to the usual rules for the mitotic block method^{11–13}. From the MI of each stage and the MT (determined in 10-d and 14-d embryos and supposed to be constant during the development of the liver) the GT and the PK for several other stages were computed.

reflect variations in k (ref. 4). Thus T_B is the minimum duration of the cell cycle and the transition probability of the hepatocytes at 5 d can be assumed to be sufficiently high that the mean generation time is close to T_B . Our value of 16 h is in reasonable agreement with estimations in other cells from α curves or FLM data^{2,7,8}. A direct estimate of T_B in this cell population from FLM data could improve the results, however, an error of several hours in the estimate of T_B would not dramatically alter the calculation of M_{co} , because PK is so low. The computation of SP_{co} according to the classical model was done for different values of the coefficient of variation of GT between sibling cells.

Table 2 Mitotic coincidence in chick embryo hepatocytes after a mitotic block: experimental and computed values for experiments on 10- and 14-d embryos

Experiments on 10-d embryo	
Parameters used for M_{co} computations	Mitotic coincidence (M_{co})
$n = 3$	Observed = 0.15 ± 0.09
$s = 1/5$	Computed $TP = 0.16$
$PK = 0.0132 \text{ h}^{-1}$	Computed C for $CV 10\% = 0.47$
$GT = 52.5 \text{ h}$	Computed C for $CV 70\% = 0.16$
$T_B = 16 \text{ h}$	
$MI(t) = 0.044 \pm 0.016$	
$t = 4.5 \text{ h}$	
Experiments on 14-d embryo	
Parameters used for M_{co} computations	Mitotic coincidence (M_{co})
$n = 3$	Observed = 0.22 ± 0.05
$s = 1/5$	Computed $TP = 0.24$
$PK = 0.0124 \text{ h}^{-1}$	Computed C for $CV 10\% = 0.61$
$GT = 55.7 \text{ h}$	Computed C for $CV 80\% = 0.24$
$T_B = 16 \text{ h}$	
$MI(t) = 0.075 \pm 0.021$	
$t = 6.0 \text{ h}$	

n (average number of adjacent cells per cell in the tissue section), s (probability of a cell to be the sibling of a given neighbour), PK (proliferation rate constant), GT (average generation time), T_B (B phase time), $MI(t)$ (mitotic index after a mitotic block), t (duration of the mitotic block). The same conditions were used for mitotic block as in Table 1. Seven experiments for the 10-d embryo and six experiments for the 14-d embryo were carried out, the $MI(t)$ and the M_{co} (observed) values are the average of each set of experiments. TP (according to the transition probability model), C (according to the classical model), CV (coefficient of variation accepted for the generation time between sibling cells).

In spite of a high variability, our measurements of M_{co} are in striking agreement with the predictions of the transition probability model (Table 2). They deviate clearly from the classical model when a reasonable coefficient of variation (such as 10%) is substituted. Only when coefficients as high as 70% or 80% are substituted, do the predicted values match the experimental ones (Table 2). In qualitative terms, our conclusion is that there is a great variability ($CV = 70\text{--}80\%$) of intermitotic times between sibling cells in the liver parenchyma of the chick embryo. This is quantitatively accountable on the new model as a consequence of a stochastic transition in the cell cycle. The high degree of variability is inconsistent with the deterministic procession of the classical cell cycle. As environmental differences should not exist between sibling cells, the loss of synchrony could, in principle, be explained in only two ways—either as a consequence of assymmetric cytoplasmic division, or by a random transition in the cell cycle.

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No evidence for foreign H-2 specificities on the EL4 mouse lymphoma

GARRIDO *et al.*^{1,2} have recently presented evidence that H-2 antisera react in an anomalous fashion with certain mouse lymphomas. They suggested that such reactions are due to the derepression of 'silent' genes in the tumours resulting in the expression of foreign H-2 antigens which are not expressed on normal tissue from the animal of origin. If correct, this theory would have vast implications both for the role of the H-2 antigens on the cell surface and for the mechanisms of leukaemogenesis. Their results, for example, indicated that the C57BL/6 (abbreviated to B6) lymphoma EL4 (H-2^b) was reactive in a complement-dependent microcytotoxicity test with four H-2 alloantisera (D-11, D-19, D-30, D-32), which according to their typing were not reactive against normal lymph node cells of an H-2^b strain. We present evidence here that anomalous reactivities against EL4 are probably not due to the derepression of foreign H-2 antigens, but are more likely to be a result of weak reactivities contained within these antisera against normal components on the lymphoid cell surfaces of B6 mice.

Normal and leukaemic cells were typed by direct and absorption cytotoxicity typing with a panel of H-2 antisera, most of which were the same as those employed by Garrido *et al.*^{1,2}. In agreement with their results, we found that eight of the H-2 antisera, which on direct typing failed to give a cytotoxic reaction with normal H-2^b cells, were

Table 1 Cytotoxic reactivity of H-2 antisera and absorption capacity of B6 lymph node cells and the EL4 lymphoma

NIH code*	Strain combination	Cytotoxicity†		(C.I.)‡ on Index strain	Absorbed with	After absorption§ EL4	C.I. on Index strain
		B6 LNC	EL4				
D-8	(B10 × A.SW) anti-B10.M	0	35	95+ (B10.M)	Spleen¶	5	95+
D-11	(DBA/2 × SJL) anti-DBA/1	0	35	95+ (DBA/1)	EL4	3	95+
					Spleen	0	77
D-12	(SWR × DBA/2) anti-DA/Sn	0	38	95+ (A.SW)	EL4	2	68
					Spleen	3	68
D-17	(D1.C × AKR.M) anti-DBA/1	0	36	95+ (DBA/1)	EL4	5	69
					Spleen	3	79
D-19	(B10.A × A.CA) anti-A.SW	20	42	64 (A.SW)	EL4	1	95+
					Spleen	4	49
D-23	(B10 × LP.RIII) anti-B10.A(2R)	0	29	95+ (B6-H-2 ^k)	EL4	0	41
					Spleen	1	60
D-25	(B10.D2 × C3H.NB) anti-B10.RIII (7INS)	0	48	95+ (DBA/1)	EL4	0	58
					Spleen	3	88
D-30	(B10.A × LP.RIII) anti-B10.AKM	14	80	95+ (DBA/1)	EL4	0	80
					Spleen	5	81
D-31	(B10 × A) anti-B10.D2	0	29	61 (BALB/c)	EL4	0	90
					Spleen	2	56
D-32	(B10.A(2R) × C3H.SW) anti-C3H	1	34	59 (B6-H-2 ^k)	EL4	1	60
					Spleen	8	44
D-28b	(B10.BR × LP.RIII) anti-B10.A(2R)	95+	95+	95+ (B6)	EL4	4	35
					B6 spleen	7	0
					EL4	0	18
					B6-H-2 ^k spleen	95+	95+

*Antisera were obtained from the NIH under contract PH 43-66-483.

†Direct testing was done by the cytotoxicity assay of Gorer and O'Gorman¹⁵ with modification by Boyse *et al.*¹⁶. All antisera were tested at a 1/10 dilution. Absorbed rabbit serum selected for low toxicity and high complement activity was prepared as a source of complement by the method of Boyse *et al.*³.

‡C.I. = cytotoxic index calculated as $(A - B)/(100 - B)$, where A = % of cells lysed by antiserum plus complement and B = % of cells lysed with complement alone.

§Absorption testing was done by incubating 1 part of $3 \times$ washed cells with 1.5 parts of diluted antiserum (1/10) for 30 min on ice with shaking.

||'Index strain' is the strain against which the antiserum was produced or a strain carrying the same private specificity. In no case did the B6 tumour seem to remove significantly more antibody to this specificity than B6 spleen.

¶Unless noted, spleens were taken from normal B6 mice.

reactive against EL4 (Table 1). However, we have also found that two of the sera previously reported as negative on normal H-2^b cells (D-19 and D-30) did react weakly with normal B6 lymph node cells. One explanation for our finding of positive reactions where negative results had been reported is that, in our cytotoxicity test, we used a more efficient complement source^{3,4}.

Several of the antisera, however, even with improved complementation, remained negative against normal cells but reacted with leukaemic ones; in each case, all of the reactivity against EL4 could be removed by absorbing the antisera with B6 spleen cells (Table 1). This same absorption procedure had little effect on the cytotoxic reactivity of these antisera against lymph node cells from the reported positive index strain (see Table 1). Thus, even though normal cells were unreactive in the direct cytotoxicity test, the more sensitive absorption test detected the expression of these antigenic specificities on both normal and leukaemic cells. Cytotoxicity-negative-absorption-positive is a well-known phenomenon in the HLA system^{5,6}.

We therefore propose that these reactions in the direct cytotoxicity test are due to one of three alternatives. First, the specificities detected in these sera may be antiviral in nature, with EL4 expressing a quantitative difference of viral antigens on its surface as compared to normal B6 tissue. Nowinski and Klein⁷ have shown that this is a likely occurrence. Second, the sera may be recognising an H-2 cross-reactivity previously unreported. Third, these sera may not be recognising H-2 differences only and may be contaminated with antibodies directed at non-H-2 or differentiation antigens. This latter possibility is becoming increasingly important on account of our recent reports that several H-2 alloantisera contain anti-TL activity⁸, that at least one Ia antiserum is contaminated with antibodies related to the *Tla* region⁹, and that several differentiation antigens are coded for by genes closely linked to, or con-

tained within, the H-2 complex¹⁰⁻¹². There is, therefore, a strong possibility that antibodies against these antigens are contained within anti-H-2 sera. Certain tumours which theoretically correspond to stages in normal lymphoid cell development^{13,14}, may consequently have increased representation of these differentiation antigens on their cell surfaces. Our results, therefore, provide no evidence for the derepression of 'silent' genes resulting in the expression of foreign H-2 antigens.

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Transplantation antigens *per se* are poor immunogens within a species

THE structure of HLA substances make it surprising that they provoke strong alloimmune responses¹⁻³. They are composed of two chains, one invariant within the species, and the other bearing a single determinant defined by monospecific HLA sera⁴, or a few determinants defined by polyspecific sera^{4,5}, and thus most closely resemble haptens on non-immunogenic carriers. If this is true generally of antigens determined by major histocompatibility systems (MHC), they could be expected to be poorly or non-immunogenic within a species. This, however, conflicts with the evidence that when present on viable tissue grafts, these antigens provoke strong humoral and cellular alloimmune responses⁶⁻⁸. But there is also evidence that their immunogenicity depends on additional factors^{9,10}. Here, we present data which reconcile these seemingly conflicting lines of evidence, by showing that the induction of primary immune responses (humoral and cellular) against MHC antigens *in vivo* depends both on the presentation of alloantigen and on a second signal provided by a live cell. Without the latter, the small quantities of MHC antigen present on conventional allografts would not induce primary immunity.

The two sets of observations which showed that MHC antigens are poorly or non-immunogenic within the species are that mouse red cells, which are known to carry H-2 antigens, fail to induce second set skin allograft rejection⁹, and that allogeneic rat platelets fail to induce primary humoral or cellular immunity against Ag-B determinants¹⁰. Our calculations indicated that 10^{-9} g of Ag-B glycoprotein when present on viable lymphocytes could induce a primary cytotoxic antibody response (titre 1/100–1/128), but that 10^{-5} g of Ag-B antigen when present on platelets failed to do so. As the primary response to MHC antigens is T-cell dependent¹¹, we suggested that Ag-B antigen by itself is insufficient to activate helper T cells. Because of the evidence implicating I-region determinants in the activation of helper T cells^{12,13}, the induction of a primary response by lymphocytes, but not by platelets was attributed to the presence of Ag-B and Ia-like determinants on the same cells. In order to analyse further the question of what makes Ag-B immunogenic, we have measured immune responses in rats injected repeatedly with 3.5×10^{-7} g of Ag-B antigen (assuming 10^5 antigen molecules per lymphoid cell¹⁴) bound to carriers, bearing or lacking Ia-like determinants.

Figure 1a and b illustrates the antibody responses of AS (Ag-B¹) rats injected with membranes prepared from August strain (Ag-B³) spleen and lymph node cells or from liver¹⁵, liposomes containing Ag-B³ alloantigen, or intact, viable, but heavily-irradiated (2,500 R) August spleen and lymph node cells. All inocula were adjusted to contain equal amounts of Ag-B determinants, based on the results of quantitative absorptions. All inocula except the liver membranes were also demonstrated by quantitative absorptions to have approximately equal amounts of rat Ia-like antigen. In contrast, the liver membranes contained Ag-B but no detectable Ia-like antigens. A striking difference in immunogenicity was observed, depending on whether the MHC antigens were presented on viable, but non-proliferating, lymphoid cells, or on membranes or artificial membranes (liposomes). Only intact spleen and lymph node cells, with or without complete Freund's adjuvant stimulated cytotoxic antibody responses in the unprimed rats; in the primed rats both Ag-B- and Ia-like antibodies appeared in recipients challenged with spleen and lymph node cell membranes, liposomes, or intact lymphoid cells. Liver membranes induced only Ag-B antibody in the primed recipients. In other experiments (not shown) tumour cell membranes bearing both Ia and Ag-B antigens behaved in an identical manner to spleen and lymph node membranes. Figure 1 also shows that repeated

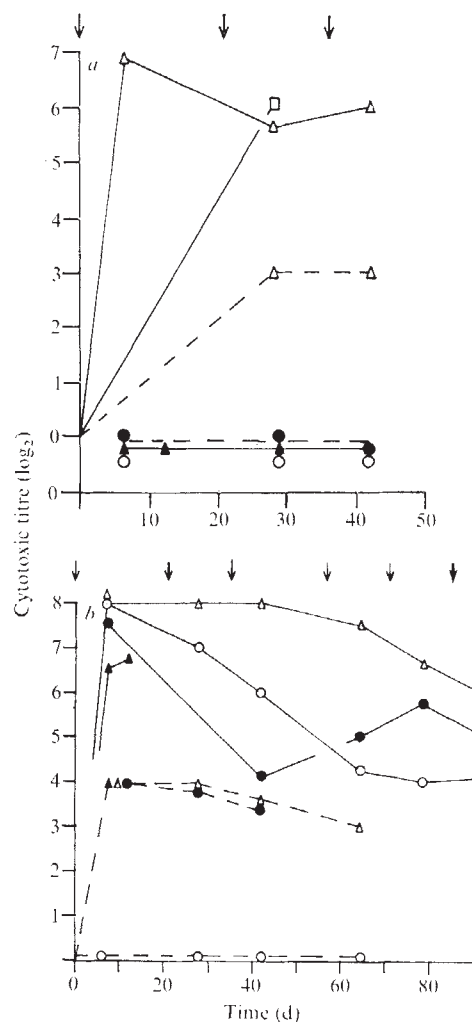


Fig. 1a, AS strain rats were injected with August strain liver membranes (○), spleen and lymph node membranes (●), liposomes bearing August strain Ag-B and Ia-like determinants (Willoughby, E., Turner, M. J. & Sanderson, A. R., in preparation) (▲), irradiated spleen and lymph node cells (2,500R) with (□), or without (△) complete Freund's adjuvant on the days indicated by the arrows. Each injection was equivalent to 3.5×10^7 August lymphoid cells in terms of Ag-B content. Animals (four per group) were tested for cytotoxic antibody titres, Ag-B response (—) being monitored against August lymph node cells and the Ia response (---) being monitored against Wistar B cells (August and Wistar share Ia but not Ag-B)¹⁰. Titres are expressed as means of each group (end point = 50% cells killed); animals in each group were either all positive or all negative. s.d., all less than 1, except for two points in b where for rats injected with spleen and lymph node membranes, the Ag-B mean titres on days 42 and 65 had s.d. of 1.1 and 1.25 respectively. **b**, As above, but rats were primed several months before these experiments and immune response was allowed to decrease to non-detectable levels before the boosts.

injections of membranes or liposomes into non-primed recipients did not succeed in exciting an antibody response; similarly (data not shown), the injection of spleen and lymph node membranes or liver membranes emulsified in complete Freund's adjuvant also failed to elicit antibody production in unprimed rats. In further experiments (not shown), DNP- or BSA- conjugated allogeneic platelets did not induce primary alloantibody responses, demonstrating that the lack of response to Ag-B was not due to the absence of a second epitope which provides essential 'help'.

Cell-mediated immunity (CMI) was measured by ⁵¹Cr release from August target cells¹⁶ incubated for 4 h with peritoneal effector lymphocytes from injected rats. August liver membranes (Table 1), and spleen and lymph node membranes or liposomes (data not shown) provoked only feeble cellular immunity in both primed and unprimed rats.

Table 1 CMI response of AS rats given August liver cell membranes

Group	Effector-target cell ratio	7	11	Day 18	28	42
Primed	100:1	4.82†	3.63	4.63†	8.57†	0.76
	50:1	3.03*	2.89	2.65	6.00†	0.52
	25:1	2.37	2.56	0.96	3.96*	0.50
Unprimed	100:1	6.77†	4.00†	2.04	10.67†	4.44
	50:1	ND	ND	0.54	6.78*	ND
	25:1	ND	ND	ND	2.69	ND
Hyperimmune	100:1	44.25†	49.26†	57.26†	62.38†	58.98†
	50:1	42.94†	45.86†	48.45†	61.23†	47.75†
	25:1	38.99†	41.82†	44.03†	56.79†	40.46†

Primed AS rats were given 2×10^7 August lymphoid cells, i.p. on day -120. Primed and unprimed AS rats were given August liver cell membranes approximately equal to 3.5×10^7 lymphoid cells, i.v. on days 0, 21 and 36. Peritoneal exudate lymphocytes taken from injected rats, on the days indicated, were tested for lysis of ^{51}Cr -August thymus cell targets. Spontaneous release (already subtracted) varied between 6 and 14% of the total count. Results are expressed as means of triplicates, those significantly above the spontaneous release level are indicated.

* $P < 0.05$; † $P < 0.01$.

Because isotope release was so low in the case of cultures containing effector cells from membrane-treated rats, no attempt was made to investigate specificity of the response at that stage. The rats were further immunised at the intervals indicated and retested for specific immunity on days 64 and 92 (Table 2). The additional injections of either liver or spleen and lymph node membranes did not lead to any substantial increase in cellular immunity. At day 92, after five spaced, boosting injections, even primed rats injected with membranes showed less than 6% specific ^{51}Cr release at an effector-target cell ratio of 100:1. This contrasts with the development of strong and specific cellular immunity in primed rats injected with intact lymphoid cells.

It is clear from these and earlier results that MHC epitopes *per se* do not have unusually powerful alloimmunogenic properties; this generalisation applies to both the classical Ag-B determinants, and the rat equivalent of Ia antigens. Our data show that to be strongly immunogenic, the antigens must be presented on viable cells, but not necessarily replicating ones, as heavily irradiated cells provide an adequate stimulus. The experiments do not show whether Ag-B or Ia alone can immunise if on a live cell, and to examine this point we have

injected allogeneic cells which either lack or have low Ia antigen content (Table 3). The results suggest that the responses to H-2 or Ag-B on viable cells does not correlate with the concentration of Ia antigen present.

The interesting point about MHC antigens is that they are strongly alloimmunogenic when present at ng levels but only when present on viable cells. In contrast, immunoglobulin allotypes which also approximate to haptens on non-immunogenic carriers require μg to mg amounts of immunogen with adjuvant in order to induce allotype antibodies. The immunogenic potency of HLA and other MHC components is also entirely different across a species barrier, but in such cases, the number of 'foreign' epitopes is such that the molecule no longer approximates to a hapten on a non-immunogenic carrier. The requirements for Ag-B to induce a primary response *in vivo*, that is, the presence of an intact nucleated stimulating cell, not necessarily capable of division, are suspiciously like those which have to be fulfilled for the generation of blasts or cytotoxic-T cells *in vitro*^{12,17}. Lafferty and Woolnough¹⁷ have proposed a two-signal activation hypothesis for immune responses to H-2 antigens. Our data fit this idea and also show that two signals are needed for activation of res-

Table 2 CMI response of AS rats given August cell membranes

Group	Injection	Effector-target cell ratio	Net. rel. v. August	Day 64 Net. rel. v. AS	Spec. rel.	Net. rel. v. August	Day 92 Net. rel. v. AS	Spec. rel.
1 (Primed)	Liver membranes	100:1	10.70	5.63	5.07	9.82	4.08	5.74
		50:1	ND	ND	ND	3.59	0.90	2.69
		25:1	ND	ND	ND	1.51	0.59	0.92
2 (Primed)	Spleen and lymph node membranes	100:1	11.81	8.70	3.11*	8.87	7.16	1.71
		50:1	8.29	5.55	2.74	ND	ND	ND
		25:1	4.24	3.71	0.53	ND	ND	ND
3 (Unprimed)	Spleen and lymph node membranes	100:1	2.87	5.07	-2.20	1.23	2.95	-1.72
		50:1	2.99	1.85	1.14	ND	ND	ND
		25:1	0.21	2.64	-2.43	ND	ND	ND
4 (Primed)	Spleen and lymph node whole cells (2,500 R)	100:1	19.59	5.51	14.08†	34.06	8.00	26.06†
		50:1	11.96	3.96	8.00†	21.85	6.61	15.24†
		25:1	6.31	2.81	3.50	15.97	5.53	10.44†

Primed AS rats were given 2×10^7 August lymphoid cells, i.p. on day -120. Groups 1, 2 and 3 were given August cell membranes approximately equal to 3.5×10^7 lymphoid cells, i.v. on days 0, 21, 36, 57, 71 and 85. Group 4 was given 3.5×10^7 August lymphoid cells (2,500 R), i.v. on days 0, 21, 36, 57, 71 and 85. Peritoneal exudate lymphocytes taken from injected rats, on the days indicated, were tested for lysis of ^{51}Cr -August and ^{51}Cr -AS (syngeneic) targets. Spontaneous release (already subtracted) varied between 6 and 14% of the total count. Results are expressed as means of triplicates. Significant specific release results are indicated.

* $P < 0.05$, † $P < 0.01$.

Table 3 Primary antibody and CMI responses after injection with viable allogeneic cells containing different amounts of Ia antigens

Injected cells	Ia content	Primary antibody response	Primary CMI response
Spleen cells	+++	+++	+++
Fc receptor-negative T cells	—	++	ND
Fibroblasts	±	+	+
Spermatozoa	±	+	++
EL4	—	+++	+++

AS strain rats were injected with August strain tissues shown, except in the case of EL4, where cultured mouse tumour cells (H-2^b) were injected into DBA/2 (H-2^d) mice. Fc receptor-negative T cells were prepared by passage of nylon-wool and G10 column purified T cells over IgG anti IgG columns. Fibroblasts were used after 8–12 subcultures; the presence of some contaminating macrophages was not excluded and therefore the presence of Ia is scored as ±.

ponses against Ia-like determinants. The presence of a second epitope alone (Ia, DNP, BSA) does not provide the additional signal, whereas intact lymphocytes do. Indeed, intact lymphocytes may provide the necessary signal to endowing amounts of any non-self cell-bound component with powerful immunogenicity.

Like allogeneic platelets, liver membranes provoked secondary antibody responses against Ag-B, but not Ia-like determinants. Spleen and lymph-node cell membranes, and Ag-B plus Ia-bearing liposomes induced secondary antibody responses against both epitopes. Secondary antibody responses therefore seem to be independent of the carrier, a fact likely to be of practical importance in clinical transplantation.

Surprisingly in view of the *in vitro* data¹⁸, the secondary cellular immune response *in vivo* does seem to depend on the carrier. In spite of repeated boosts, minimal CMI was induced except by heavily irradiated, intact lymphocytes.

Our experiments show therefore that induction of primary immune responses (humoral and cellular) against MHC antigens *in vivo* depends both on the presentation of alloantigen and on a second signal. Without this, the small quantities of MHC antigen present on conventional allografts would not induce primary immunity. The second signal is not simply a second epitope, such as Ia. It is provided by intact, viable lymphocytes which need not be capable of division. Induction of secondary humoral responses does not require the second signal, the presence of alloantigen only being sufficient.

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Ionic channel formation in a living cell membrane

THERE have been several attempts to study cell membrane formation by the use of protoplasmic droplets extruded from plant cells into appropriate solutions¹. The formation of a membrane on the surface of such droplets has been confirmed by optical, electrical and other measurements^{2–4}, but some properties of these unnatural membranes have been questioned⁵. I report here experiments which indicate the possibility of observing membrane formation in a living cell. The method was based on the sealing effect^{6–8} which isolates a microelectrode tip from protoplasm into which it has been inserted.

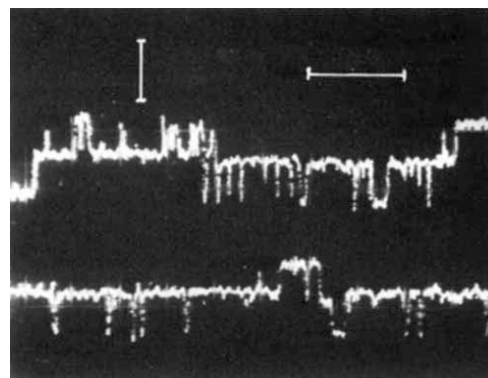


Fig. 1 Record of current obtained with a membrane containing two channels. The lowest part of each trace indicates the level of zero current. Membrane potential was -75 mV. The test microelectrode contained 100 mM LiCl, 2 mM CaCl₂. Horizontal bar represents 0.2 s, vertical bar 4×10^{-12} A.

Single cells of the freshwater alga *Nitellopsis obtusa* were used. A small portion of the cell was voltage clamped in an arrangement similar to that of the 'double sucrose gap' technique⁹. The potential of the plasmalemma was controlled by means of a glass microelectrode filled with 1 M KCl connected to the clamp amplifier. This microelectrode did not usually seal during the several hours needed for the experiment to be completed. Another microelectrode (to be sealed) with a very thin tip (about 1 μ m across) was inserted not more than 10 μ m into the protoplasm. In most cases this test microelectrode was filled with one of the following solutions: 100 mM KCl, 2 mM CaCl₂; 100 mM NaCl, 2 mM CaCl₂; 100 mM LiCl, 2 mM CaCl₂; 50 mM CaCl₂. After the test microelectrode had become sealed, its potential was small compared with the previously measured resting potential, varying from -5 mV to -15 mV. This new test microelectrode potential was clamped by means of an additional feedback amplifier (feedback resistance was equal to $3 \times 10^9 \Omega$). The changes in the current flowing through the test microelectrode occurred as voltage impulses at the output of this amplifier, and were recorded at different plasma-

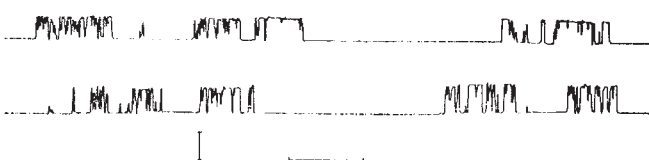


Fig. 2 Records of current showing inactivation intervals. One channel was present. Current did not always reach maximum value because of the limited response speed of the pen recorder. Upper record, membrane potential -75 mV. Lower record, membrane potential -65 mV. Test microelectrode solution: 50 mM KCl, 50 mM NaCl, 2 mM CaCl₂. Horizontal bar represents 6 s, vertical bar 10^{-12} A.

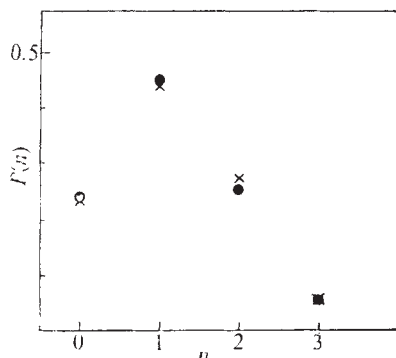


Fig. 3 Probability distribution ($P(n)$) over different states of a system of three channels. Experimental data are depicted by full circles. Crosses represent probability values calculated if the elementary probabilities of the open and closed states of a single channel (p (0.382) and q (0.618), respectively) were assumed. n is the number of channels open simultaneously. The test microelectrode contained 50 mM CaCl_2 . Membrane potential -85 mV.

lemma potentials. Each membrane potential given here is the difference between the plasmalemma potential and that of the test microelectrode. Figure 1 shows that the current fluctuated between several equally spaced levels, indicating that several identical conducting units were involved. From this I conclude that the steps in the current arise from separate ionic channels operating in the area of membrane formed around the tip of the test microelectrode during sealing. This conclusion is confirmed by the results described below.

Ionic channels occurred only if the test microelectrode was filled with an appropriate solution. In general, sealing alone occurred with any solution in the microelectrode, but calcium ions, at least in millimolar concentrations, were needed for channels to form. This is consistent with the well established fact that calcium ions are necessary for the normal functioning of living membranes.

The conductance of a single channel was determined with different solutions in the test microelectrode. With all the solutions mentioned above the slope conductance was equal to $(0.9\text{--}1.2) \times 10^{-11} \Omega^{-1}$, regardless of the cation present. When the current with one channel present was recorded for a longer time, characteristic trains of short impulses were observed, separated by relatively long intervals when the channel did not conduct (Fig. 2). This suggests that a three-state kinetic scheme is appropriate for such a channel, with open, closed and inactivated states. The kinetic properties of these channels are also voltage dependent—the more depolarised the membrane, the more frequently the channel switches on.

The probabilities of the simultaneous occurrence of a given number of channels in the conducting state were determined from long-term records obtained with many-channel membranes. They are given in Fig. 3 as a function of the number of open channels for the area of membrane containing three channels. The calculated points represent the binomial probability distribution obtained when appropriate probabilities were assumed for the open and closed states. The agreement between these two sets of points indicates that the channels act independently.

These results indicate that sealing is caused by the formation of the plasmalemma around the tip of the microelectrode. The method described here offers the possibility of further investigation, at the submicroscopic level, of membrane formation *in vivo*.

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Sensitisation of pituitary cells to luteinising hormone releasing hormone by clomiphene citrate *in vitro*

SINCE the original study of Greenblatt¹ in 1962, clomiphene citrate (2 [P-(2-chloro-1, 2-diphenylvinyl) phenoxy] triethylamine dihydrogen citrate) (Clomid) has been used successfully to induce ovulation in anovulatory conditions. The ovulation inducing effect of Clomid is mediated through a transient increase in the pituitary release of luteinising hormone (LH) and follicle stimulating hormone (FSH), which in turn initiates follicular maturation and ovulation^{2,3}. Although the mechanism of action of Clomid is unknown, this substance has been reported to possess both antioestrogenic and oestrogenic properties. Experimental studies of the pituitary, hypothalamus and uterus have shown that Clomid competitively inhibits oestradiol binding to oestrogen receptors, suggesting an antioestrogenic action^{4–7}. On the other hand, the observation that Clomid stimulates uterine growth is suggestive of an oestrogenic action^{8,9}. Oestradiol has been shown to increase the responsiveness of cultured pituitary cells to luteinising hormone releasing hormone (LHRH)¹⁰. We show here, using cultured pituitary cells as a model system, the possible antioestrogenic and oestrogenic effects of Clomid on LH release *in vitro*, and demonstrate that Clomid, like oestradiol-17 β , increases the responsiveness of gonadotrophs to LHRH *in vitro*. We conclude that Clomid acts as an oestrogen rather than an antioestrogen on the pituitary gonadotrophs.

Anterior pituitary cells were prepared from ovariectomised rats and cultured *in vitro* for 2 d in the presence or absence of Clomid (the Clomid used contained an equal amount of enclomiphene citrate and zuclophene citrate) and/or oestradiol-17 β . At the end of the culture period, the pituitary cells were washed and then reincubated for 3 h with increasing concentrations of LHRH. The LH accumulation in the media during the 3 h incubation was measured by radioimmunoassay. As shown in Fig. 1, pituitary cells pretreated with 10^{-8} M oestradiol-17 β were more responsive to LHRH when compared to control cultures. A dose-dependent increase in LH production was detected with increasing concentrations of LHRH in both control and oestradiol-treated cells, and in both cases 10^{-7} M LHRH elicited a maximal response. Compared to the basal level, an increase in LH production was observed with 10^{-10} M LHRH in control cultures and with 10^{-11} M LHRH in oestradiol-treated cultures. The half maximal effective dose (ED_{50}) of LHRH was 3×10^{-9} M and 1×10^{-10} M in control and oestradiol-treated cultures, respectively. These observations demonstrate that oestradiol increases the sensitivity of pituitary gonadotrophs to LHRH *in vitro*.

When pituitary cells were treated with Clomid (10^{-8} M), a similar increase in LHRH responsiveness was observed (Fig. 2). Compared to control cultures, Clomid lowered the ED_{50} of LHRH from 3×10^{-9} M to 1×10^{-10} M. A similar decrease in ED_{50} was also observed when the pituitary cells were incubated with a combination of oestradiol and Clomid (Fig. 2). As enclomiphene citrate (formerly called *cis*-clomiphene citrate) is believed to be the active component in the Clomid mixtures, the effect of this isomer on cultured pituitary cells was tested and was found to be as effective

as the Clomid mixture (unpublished data).

The present observations have shown that oestradiol increases the responsiveness of gonadotrophs to LHRH, thus confirming the finding of Drouin *et al.*¹⁰. Furthermore, we found that Clomid causes an increase in LHRH-induced LH production similar to that produced by oestradiol. Taken together, these observations suggest that Clomid acts as an oestrogen rather than an antioestrogen on the pituitary gonadotrophs. The observation that Clomid increased pituitary gonadotrophin release suggests an oestrogenic sensitisation of pituitary gonadotrophs to LHRH. Such an increase in pituitary sensitivity could be important in

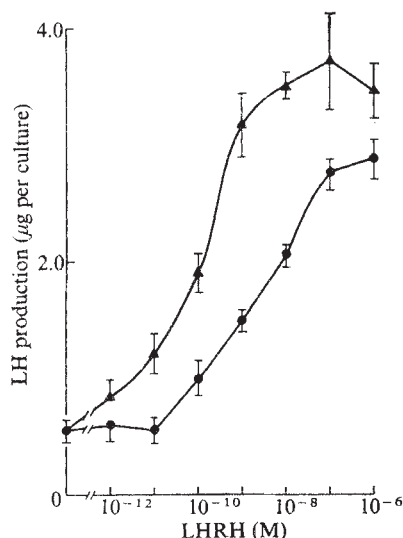


Fig. 1 Effect of oestradiol treatment on LHRH-induced LH release by cultured anterior pituitary cells. Adult female Sprague-Dawley rats (70–80 d of age) were ovariectomised by Hormone Assay Co. (Chicago), and killed 2 to 3 weeks later. Pituitary cell suspensions were prepared in HEPES buffer (137 mM NaCl, 5 mM KCl, 0.7 mM Na_2HPO_4 , 25 mM HEPES, 10 mM glucose and 360 μM CaCl_2 at pH 7.2) by modification of the enzyme dispersion method originally described by Vale *et al.*¹⁶. After removing the intermediate and posterior lobes, the anterior pituitaries were cut into eight pieces and dispersed by an enzyme solution containing 0.4% collagenase (Worthington; 144 units mg^{-1}); 10 $\mu\text{g ml}^{-1}$ DNase (from bovine pancreas, 2,100 units mg^{-1} ; GIBCO) and 1% bovine serum albumin (Sigma). The pituitary pieces were incubated at 37 °C for 1.5 h into a cell suspension by repeated pipetting with graded series of micropipettes at 30-min intervals. At the end of the incubation period, the pituitary cells were collected by centrifugation (250g for 5 min), washed five times with HEPES-0.1% BSA buffer, and resuspended into McCoy's 5a medium (modified:GIBCO). Viable cells ($\geq 90\%$) were determined by counting cells which excluded Trypan blue stain. Pituitary cells ($1\text{--}2 \times 10^5$) were cultured in 35×10 mm tissue culture dishes (Falcon) in a total of 2 ml of McCoy's 5a medium (modified) supplemented with L-glutamine (2 mM), penicillin (100 U ml^{-1}), streptomycin sulphate (100 $\mu\text{g ml}^{-1}$), 10% horse serum and 2.5% foetal calf serum. Cultures were maintained in a 95% air–5% CO_2 incubator at 37 °C. The serum was pretreated with charcoal to absorb free steroid as described earlier¹⁰. Oestradiol-17 β in 25 μl of McCoy's medium were added to the incubation medium to obtain a final concentration of 10^{-8}M , and pituitary cells were incubated for 2 d. After the treatment, cells were washed four times with 2 ml of medium 199 (GIBCO), reincubated for 3 h in McCoy's media containing various concentrations (10^{-12} – 10^{-6}M) of synthetic LHRH. After the test period, media were collected, diluted with phosphate buffer (0.02 M PO_4 ; 0.15 M NaCl, 0.05 M EDTA) containing 0.1% gelatin and stored at -20°C until assayed. Concentration of LH in incubation medium was determined by double antibody radioimmunoassay, employing reagents provided by Dr A. F. Parlow. Results are expressed in terms of the reference standards NIAMDD rat LH-RP-1. LH production by pituitary cultures are shown as mean $\pm$ s.e. for control (●) and oestradiol treated (▲) cultures. Three to four culture dishes were used for each datum point, with duplicate determinations for each culture.

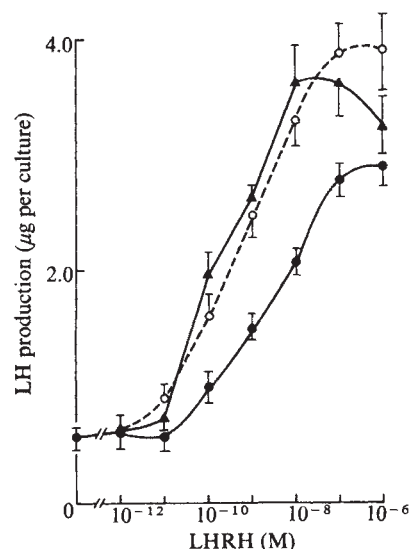


Fig. 2 Effects of Clomid and Clomid plus oestradiol on LHRH-induced LH release *in vitro*. Experimental procedures are described in Fig. 1. Anterior pituitary cells were incubated for 2 d in media (control group ●), media containing 10^{-8}M Clomid (▲) or media containing 10^{-8}M Clomid plus 10^{-9}M oestradiol-17 β (○). These data are expressed as mean $\pm$ s.e. Three or four culture dishes were used for each datum point with duplicate determinations for each culture. Similar data were obtained in three separate experiments.

initiating the increase of circulating LH observed in anovulatory women following Clomid treatment.

Clomid and other related nonsteroidal compounds, such as nafoxidine, have traditionally been considered to be oestrogen antagonists^{4–7}. If Clomid had been acting as an antioestrogen in the pituitary cells, then we would have expected it to antagonise the oestradiol-induced sensitisation of gonadotrophs; however, no antagonistic effect was observed when these cells were treated with a combination of Clomid and oestradiol (Fig. 2). It is therefore possible that Clomid acts as an oestrogen in the pituitary cells. The oestrogenic property of Clomid has been reported before, one injection of Clomid in rats caused sustained uterine growth which was accompanied by a prolonged accumulation of oestrogen receptors in uterine nuclei^{8,9}. If it causes similar changes in oestrogen receptor dynamics in the pituitary, Clomid may sensitise pituitary gonadotrophs to LHRH-induced LH release through binding and retention of oestrogen receptors.

In addition to its effect on the pituitary, the action of Clomid on other oestrogen target tissues, especially the ovary and the hypothalamus, must be considered. No conclusive data on the direct effect of Clomid on ovarian function are available, although an effect on ovarian steroidogenesis has been suggested^{11,12}. In connection with the hypothalamus, oestrogenic compounds were shown to stimulate the formation of adenosine-3'5'-monophosphate (cyclic AMP) in hypothalamus incubated *in vitro*, and this oestrogen-induced formation of cyclic AMP can be blocked by Clomid^{13–15}, indicating an antioestrogenic action of Clomid on the hypothalamus. These findings suggest that Clomid may modulate LHRH release by the hypothalamus, and any changes in LHRH output could further modify pituitary sensitivity to this hormone. Although the analyses of the isolated pituitary unit may not reflect the complex hypothalamic–pituitary–ovarian interactions *in vivo*, the use of cultured pituitary cells in this report offers the distinct advantage of examining the direct effect of oestrogen and Clomid on the pituitary gonadotrophs. In these experimental conditions, the present observations provide the first direct evidence that Clomid sensitises pituitary gonadotrophs to LHRH action.

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Receptor basis for dopaminergic supersensitivity in Parkinson's disease

In patients with Parkinson's disease, the concentration of dopamine in the basal ganglia of the brain is markedly reduced in accordance with the degeneration of the nigrostriatal dopamine-containing neurones^{1,2}. This fact provided the basis for the successful clinical introduction of L-dopa (L-3,4-dihydroxyphenylalanine) for Parkinson's disease^{3,6}. It has been suggested that one of the critical factors compensating for the loss of dopamine neurones may be the development of "denervation supersensitivity" in the striatum, as severe cases react more sensitively to L-dopa than milder cases or controls⁷⁻⁹. By measuring dopamine receptors in the putamen and caudate of *post-mortem* brains from Parkinson patients, we report here evidence in support of the theory of dopaminergic supersensitivity in Parkinson's disease.

The average age (and age range) for the 14 neurologically normal patients was 43 yr (19-77 yr), whereas that for the six non-L-dopa-treated Parkinson patients was 67 yr (58-76 yr). The interval between death and freezing of the brain was 6-33 h (mean, 17 hr) for controls, and 4.5-23 h (mean, 15 h) for the Parkinson brains. The cause of death in the control subjects was either a sudden accident (for example, car accident) or myocardial infarction; that for the Parkinson patients, however, was pneumonia.

The specific binding of ³H-haloperidol¹⁰⁻¹⁷ and of ³H-apomorphine^{18,19} served to label the brain dopamine receptors. Frozen samples of *post-mortem* human brain tissue were thawed and suspended in buffer (1 ml per 40 or 50 mg tissue). The buffer contained 15 mM Tris-HCl, pH 7.4, 5 mM Na₂ EDTA, 1.1 mM ascorbic acid and 12.5 μM nialamide. After homogenisation in a glass-Teflon homogeniser, the 1-ml sample was preincubated for 1 h at 37 °C and then frozen at -20 °C. For the radioreceptor assay, the homogenate was thawed, resuspended, and centrifuged at 39,900g for 15 min at 4 °C. The pellet was resuspended in 3 ml buffer, and homogenised with a Polytron PT-10 at

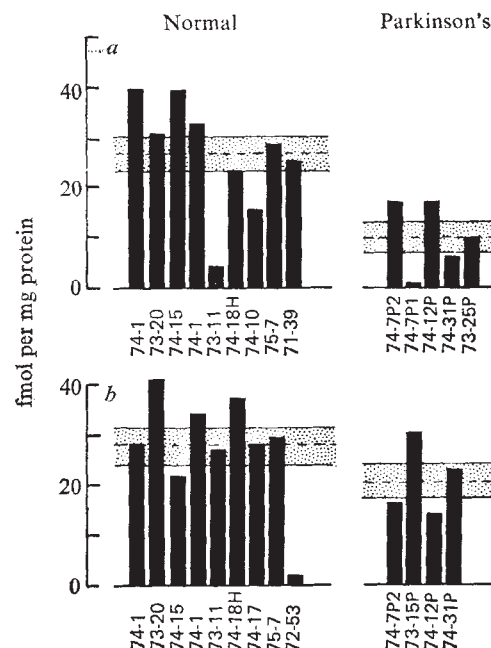
Table 1 Dopamine and homovanillic acid content in Parkinson's striatal tissues

	Dopamine (μg g ⁻¹)		Homovanillic acid (μg g ⁻¹)	
	average	(range)	average	(range)
Control putamens	5.9	(2.5-9.8)	10.7	(6.5-20.8)
Parkinson putamens (No L-dopa therapy)	0.4	(0.35-0.41)	2.5	(1.9-3.4)
Parkinson putamens (L-dopa treated)	0.5	(0.4-0.7)	9.2	(2.6-19.0)
Control caudates	4.5	(2.1-7.2)	5.8	(3.9-8.4)
Parkinson caudates (No L-dopa therapy)	1.2	(0.8-2.0)	2.0	(1.5-3.0)
Parkinson caudates (L-dopa treated)	2.1	(0.8-4.3)	7.7	(3.4-15.5)

a setting of 5.5 (full-scale=10) for 20 s. Aliquots (0.2 ml) of this final homogenate were added to 12×75 mm tubes containing 0.2 ml of ³H-apomorphine (14.1 Ci mmol⁻¹; final concentration of 3.0-3.3 nM) or 0.2 ml of ³H-haloperidol (7.8 Ci mmol⁻¹; 1.7 nM final concentration) and 0.2 ml of buffer with or without (+)-butaclamol (the final concentration of which was 100 nM for the ³H-haloperidol assay, and 1 μM for the ³H-apomorphine assay). The final concentrations of the ³H-ligands were chosen near their K_D values¹⁰. Specific binding was defined as the total binding minus that which occurred in the presence of (+)-butaclamol. Putamen and caudate assays were repeated from 3 to 16 times (usually 6 times), each assay in sextuplicate. The dopamine²⁰ and homovanillic acid²¹ contents were also measured. The average values (and ranges) for dopamine and homovanillic acid contents in the tissues (in μg g⁻¹ tissue) are listed in Table 1.

The specific binding of ³H-apomorphine (Fig. 1) was significantly reduced in the putamen. This observation is in accord with the recent finding that 6-OH-dopamine-induced lesions of the nigral cells (in rats) reduced the specific

Fig. 1 The specific binding of ³H-apomorphine in the putamen (a) and caudate (b) of post-mortem brains from Parkinson patients was lower than normal. Each column indicates the average of 3-16 (usually 6) separate assays (in sextuplicate) for each patient's brain region. The horizontal lines, broken and solid, indicate the mean and s.e.m. respectively, for each group. None of the patients had been on L-dopa therapy. ($P < 0.001$ for putamen).



binding of ^3H -apomorphine in the striatum by 60% (J. Nagy *et al.*, unpublished). Thus, since a large proportion of specific ^3H -apomorphine binding occurs to presynaptic dopamine receptors (J. Nagy *et al.*, unpublished), it is reasonable to assume that ^3H -apomorphine binding is reduced in Parkinson's disease because of the nigral cell degeneration^{1,2}.

In contrast to ^3H -apomorphine, there was a significant increase in specific ^3H -haloperidol binding in the putamens of Parkinson patients who did not receive any L-dopa therapy (Fig. 2). The specific binding of ^3H -haloperidol was normal, however, in the tissues from patients who had been taking L-dopa for years. The average daily dose of L-dopa was 3.3 g (range, 1.5–6 g). The last dose before death averaged 0.6 g (0.3–1 g) and had generally been administered 30 h (1.8–98 h) before death.

This marked increase in specific ^3H -haloperidol binding is also in agreement with animal data. Lesions of the nigral cells (or axon tracts) in rats lead to an increased specific binding of ^3H -haloperidol in the striatum (ref. 22 and J. Nagy *et al.*, unpublished).

The increase in specific ^3H -haloperidol binding in the striatal tissue of the non-dopa-treated cases presumably reflects an increased number (or increased affinity constant) of postsynaptic dopamine receptors, supporting the notion of denervation supersensitivity in Parkinson's disease⁷⁻⁹. This relation between dopaminergic supersensitivity and higher ^3H -haloperidol binding is similar to that seen after chronic neuroleptic treatment; animals or patients put on long-term neuroleptic medication revealed an increased number of ^3H -haloperidol receptors²³⁻²⁷ and an increased behavioural sensitivity to dopamine-mimetic drugs²⁸⁻³⁰.

It is interesting to note that ^3H -haloperidol binding was normal in those patients who had been on long term L-dopa therapy. This agrees with the findings of Reisine *et al.*²⁴ who found that the amount of specific ^3H -spiroperidol binding of L-dopa-treated Parkinson's patients was normal in the putamen and somewhat below normal in the caudate and the globus pallidus. This suggests that L-dopa might have 'desensitised' the dopamine receptors and probably reverted the dopaminergic supersensitivity of the striatum. The normal values for specific ^3H -haloperidol binding here agree well with the normal values reported by Enna *et al.*³². In fact, it has recently been observed that high doses of L-dopa can desensitize reserpine-induced hyperdopaminergic behaviour³⁰ and can reduce the elevated amount of specific ^3H -dopamine binding seen in animals made supersensitive by haloperidol³³. Although the trend of the findings in the caudate were the same as those for the putamen, the actual values did not quite achieve a level of significance of $P < 0.05$ (standard two-tailed Student's *t* test). This is in general accord with past observations that the neuropathology within the putamen is generally more severe than that in the caudate (see Table 1 and refs 1–3). A possible source of complication might be that the cause of death may itself affect dopamine receptors. The Parkinson patients died of pneumonia, whereas the control group died suddenly. There is some evidence, however, that pneumonia does not affect dopamine receptors (our unpublished observations); in addition, although the untreated and L-dopa-treated patients all died of pneumonia, they differed in the amount of ^3H -haloperidol binding.

In conclusion, the finding of increased specific ^3H -haloperidol binding in the putamen of untreated Parkinson's disease patients supports the notion⁷⁻⁹ of denervation supersensitivity as one of the factors compensating the disease. The data from the L-dopa-treated patients indicate that long-term L-dopa medication removes this compensating feature. This desensitisation of the striatal dopamine receptors may represent a major limiting factor for the chronic high dose use of L-dopa. In addition, the transient drop of dopamine receptor sensitivity to below the normal

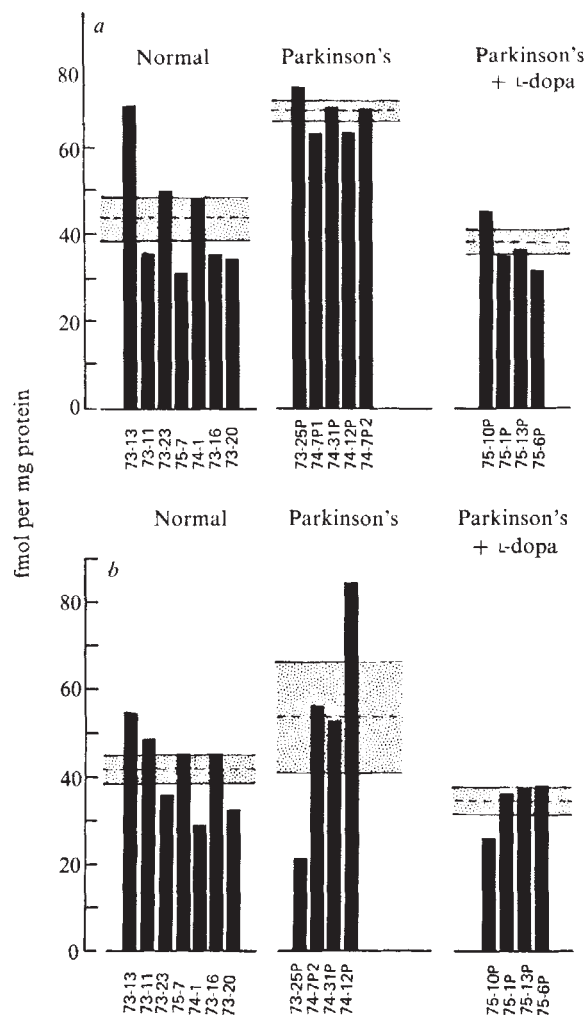


Fig. 2 ^3H -Haloperidol binding in putamen (a) and caudate (b) of normal, Parkinson and L-dopa-treated Parkinson brains. Evidence for an increase in the specific binding of ^3H -haloperidol in the putamen of brains ($P < 0.001$) from Parkinson patients who had not received L-dopa; a non-significant increase occurred in the Parkinson caudate. The putamen and caudate of all L-dopa-treated patients gave normal binding values. Horizontal lines indicate mean (---) and s.e.m. (—)

levels also may play a part in the 'on-off' phenomenon which is frequently seen in patients taking L-dopa chronically. Such observations may suggest a paradoxical treatment to patients who become refractory to L-dopa; removing or substantially reducing their L-dopa medication, while controlling the clinical condition with alternate non-dopaminergic medications, may restore their supersensitive receptors as well as reactivity to L-dopa.

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An endogenous substrate for cGMP-dependent protein kinase in mammalian cerebellum

CYCLIC guanosine monophosphate (cGMP)-dependent protein kinases, a class of protein kinases which are activated by low concentrations of cGMP, but not of cyclic adenosine monophosphate (cAMP), have been demonstrated in a number of invertebrate^{1,2} and vertebrate^{3–11} tissues. In contrast to cAMP-dependent protein kinases, for which numerous substrates have been found, it has been difficult to demonstrate the existence of endogenous substrates for cGMP-dependent protein kinases. Until the present investigation, endogenous substrates for cGMP-dependent protein kinases had been identified in only two types of tissue, smooth muscle⁵ and intestinal brush border epithelium¹⁰; in both tissues the substrates were found only in membrane fractions. Experimental evidence suggests that cGMP may play a part in neuronal function^{12–14}. Although a cGMP-dependent protein kinase has been found in the cytosol of mammalian cerebellum^{3,4,7}, no endogenous substrate has previously been found in this enzyme. We report here the presence in the cerebellum of a soluble protein specifically phosphorylated by the cGMP-dependent protein kinase of that tissue.

In order to search for a possible substrate for the cGMP-dependent protein kinase present in the cytosol of mammalian cerebellum, endogenous phosphorylation of cerebellar cytosol was examined in the presence of cGMP and cAMP. The addition of $1 \mu\text{M}$ cGMP to the soluble fraction resulted in the phosphorylation of a protein with an apparent molecular weight of 23,000 (heavy arrow, Fig. 1). The phosphorylation of this protein was rapid, reaching half-maximal in approximately 15 s. An equal concentration of cAMP failed to stimulate the incorporation of [³²P]-phosphate into this protein. The observation that cAMP stimulated phosphate incorporation into several proteins of lower and higher molecular weight (light arrows, Fig. 1) indicates that cAMP-dependent protein kinase was active in the cytosol preparation.

The results shown in Fig. 1 indicated that the 23,000 MW protein was a substrate for the cGMP-dependent protein

kinase rather than for the cAMP-dependent protein kinase present in cerebellar cytosol. This conclusion was supported by studies in which preparations of cGMP-dependent protein kinase and type II cAMP-dependent protein kinase were partially purified from rabbit cerebellum and added to cerebellar cytosol. (There is very little type I cAMP-dependent protein kinase in this tissue (unpublished observations).) The effect of various concentrations of cGMP and cAMP on the phosphorylation of the 23,000 MW protein in the presence of added cGMP-dependent protein kinase is shown in Fig. 2. Cyclic GMP caused a half-maximal phosphorylation of the 23,000 MW protein at a concentration of 60 nM. (The K_a value for cGMP of the homogenous cGMP-dependent protein kinase from bovine lung was 22 nM using histone f_{2b} as a substrate²⁰.) When an equal number of units of type II cAMP-dependent protein kinase was added to the cytosol, only a slight increase in the phosphorylation of the

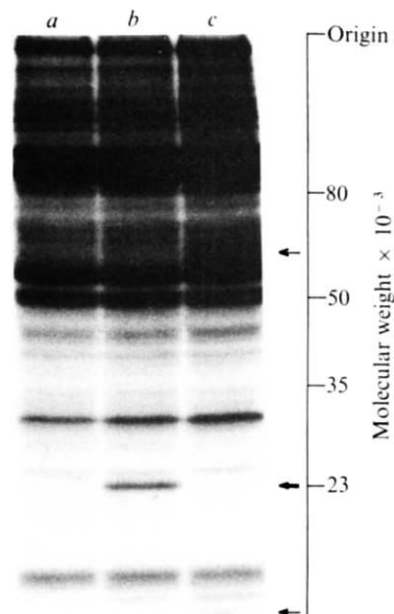


Fig. 1 Endogenous protein phosphorylation in cerebellar cytosol. Cerebellar tissue from young, male New Zealand White rabbits (9 kg) was homogenised in two volumes of homogenisation buffer containing 20 mM Tris, 25 mM β -mercaptoethanol, 0.25 M sucrose, 0.025 mM phenylmethylsulphonyl fluoride (a protease inhibitor) and 2.5% ethanol (required for solubilisation of the phenylmethylsulphonyl fluoride), pH 7.5. After centrifugation of the homogenate for 1 h at 100,000g, the resulting supernatant was passed over a Sephadex G-25 column to remove endogenous adenosine triphosphate (ATP) and cyclic nucleotides. This extract was termed the 'cytosol'. The incubation mixture for the protein phosphorylation reaction contained, in a final volume of 100 μl , 50 mM glycerol phosphate (pH 7.5), 0.3 mM EGTA, 2 mM theophylline, 10 mM MgCl_2 , 30 μM [γ -³²P]ATP (2×10^7 d.p.m. nmol^{-1}), 175 μg of cytosol protein and 1×10^{-6} M cGMP or cAMP, as indicated. The reaction was initiated by addition of [γ -³²P]ATP and was carried out for 1.5 min at 30 °C. The reaction was terminated by the addition of a sodium dodecyl sulphate (SDS) 'stop solution'¹⁵ and the samples heated at 100 °C for 1 min. The entire sample was subsequently applied to a 12% polyacrylamide slab gel and electrophoresis carried out using a procedure modified from Maizel¹⁶, as described elsewhere¹⁵. Gels were then stained for protein and dried, and autoradiography was performed¹⁷. Cyclic GMP, but not cAMP, stimulated the phosphorylation of a protein with an apparent molecular weight of 23,000, as indicated by the heavy arrow. SDS-gel electrophoresis, using several different acrylamide concentrations, revealed only one band into which phosphate incorporation was stimulated by cGMP, suggesting that this band corresponds to a single phosphoprotein. No specific stimulation by cGMP of phosphate incorporation into other proteins was observed, even when electrophoresis was carried out on a 7.5% polyacrylamide gel, which increased the separation of higher molecular weight proteins. Using procedures published elsewhere¹⁸, all radioactive bands shown in this autoradiograph were found to be due to incorporation of [³²P]-phosphate into protein rather than into lipid or nucleic acid. a, Control; b, $+1 \times 10^{-6}$ M cGMP; c, $+1 \times 10^{-6}$ M cAMP.

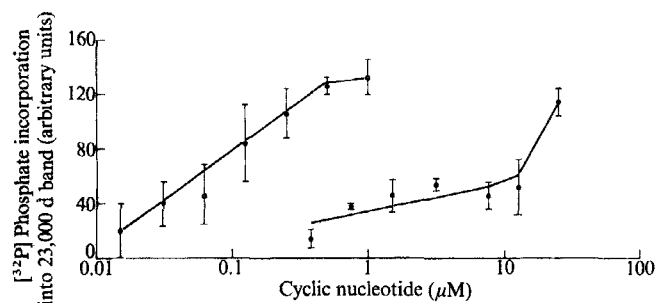


Fig. 2 Effect of various concentrations of cGMP (○) and cAMP (●) on the phosphorylation by cGMP-dependent protein kinase of the 23,000 MW band present in cerebellar cytosol. The incubation mixture contained, in a final volume of 100 μl, 50 mM glycerol phosphate (pH 7.5), 0.3 mM EGTA, 1 mM 3-isobutyl-1-methylxanthine, 10 mM MgCl₂, 15 μM [γ -³²P]ATP (2×10^7 d.p.m. nmol⁻¹), 75 μg of cytosol protein, 2.5 units of cGMP-dependent protein kinase and the indicated concentrations of cGMP and cAMP. (Cyclic GMP-dependent protein kinase and type II cAMP-dependent protein kinase were partially purified from rabbit cerebellum by the following procedure. Tissue was homogenised in six volumes of the homogenisation buffer described in the legend to Fig. 1, and the homogenate centrifuged at 25,000g for 1 h. The resulting supernatant was applied to a DEAE-cellulose column and eluted with a linear gradient of 0 to 0.35 M NaCl in the homogenisation buffer. Column fractions were assayed for cGMP- and cAMP-dependent protein kinase activities by the method of Witt and Roskoski¹⁸, and the peak fractions of each enzyme were separately pooled and concentrated by pressure dialysis. One unit of protein kinase activity is defined as 1 pmol per min of phosphate transferred to histone type IIA (Sigma).) Incubation was for 1 min at 30 °C. Other experimental procedures were as described in the legend to Fig. 1. Incorporation of radioactive phosphate was measured quantitatively by densitometric analysis of the autoradiograms as described elsewhere¹⁷. Phosphorylation of the 23,000 MW protein was calculated as the increase above that observed in the absence of added nucleotide, which amounted to 87 arbitrary units. The data points represent the individual and mean values for two determinations.

23,000 MW protein occurred (data not shown). Similarly, in the experimental conditions shown in Fig. 2 (reduced concentrations of ATP and cytosol protein), cGMP and cAMP had little or no effect on phosphate incorporation into the 23,000 MW band in the absence of added protein kinase.

In another series of experiments, type I and type II cAMP-dependent protein kinases, as well as cGMP-dependent protein kinase, were prepared from cytosol of bovine pulmonary arteries, using procedures similar to those described in the legend to Fig. 2 for the protein kinases from cerebellar cytosol. The cGMP-dependent protein kinase partially purified from bovine smooth muscle, when added to cerebellar cytosol fractions, stimulated the phosphorylation of the 23,000 MW protein; in contrast, the type I and type II cAMP-dependent protein kinases from smooth muscle failed to increase significantly the amount of phosphate incorporated into the cerebellar cytosol protein.

In the experimental conditions shown in Fig. 2, the phosphorylation of a 23,000 MW protein band was observed only in cerebellum. The partially purified cGMP-dependent protein kinase from bovine pulmonary arteries was added to cytosol from several different regions of rabbit brain including caudate, hippocampus and cerebral cortex without revealing the phosphorylation of such a protein. The absence of cGMP-stimulated protein phosphorylation in those regions of the brain does not seem to be due to the presence of an endogenous inhibitor of the cGMP-dependent protein kinase, as cytosol from caudate, hippocampus or cerebral cortex, when added to cerebellar cytosol, did not inhibit the endogenous phosphorylation of the 23,000 MW protein band in the cerebellar cytosol.

Thus, the soluble protein described here seems to be a specific substrate for a cGMP-dependent protein kinase

rather than for a cAMP-dependent protein kinase. Its non-uniform distribution in the nervous system suggests a specific functional role for this protein in cerebellum. Elucidation of this function may provide a model for understanding, in more general terms, the role of cGMP in neuronal function.

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Reversible loss of calcium control of tension in scallop striated muscle associated with the removal of regulatory light chains

THE control by calcium of the ATPase activities of myosin, actomyosin and myofibrils from molluscan muscles requires the presence of a particular 'regulatory' light chain of myosin^{1,2}. In these muscles troponin is not found in stoichiometric amounts and does not function as a major regulatory component^{2,3}. In scallop muscles one of the two regulatory light chains per myosin is readily and reversibly removed by reducing the divalent cation concentration with EDTA^{2,4}, with the result that the preparations become 'desensitised' to calcium, that is, the ATPase activity is maximal whether or not calcium is present. 'Resensitising' can be achieved by the recombination of regulatory light chains with myosin or myofibrils. We have extended these studies to a scallop muscle preparation (chemically 'skinned' fibre bundles) in which the contractile machinery is intact, and we show here that the regulatory light chains can be removed and recombined, with a concomitant loss and recovery of calcium control over tension production.

Preparations of a suitable size for mechanical experiments were usually obtained by placing a strip of muscle in a relaxing solution (Table 1) at room temperature (20–25 °C) and subdividing the strip into fibre bundles about 50–200 μm thick and 50–500 μm wide. In some experiments individual fibre bundles were subjected to chemical skinning and subsequent treatment entirely on the mechanical apparatus, and these bundles were in some cases later analysed for light chain content by urea gel electrophoresis. Larger quantities of muscle were required for actomyosin ATPase measurements, and in these experiments a number of bundles were treated together, and samples were taken at various stages of the procedure for parallel mechanical and biochemical tests.

The procedure in a complete mechanical experiment was as follows. The bundle was chemically skinned to remove

Table 1 Experimental solutions

	Relaxing	Preactivating	Activating	Quick relaxing	Rigor	Desensitising
KCl	50	50	50	50	50	40
EGTA	5	0.1	—	50	5	—
CaEGTA	—	—	5	—	—	—
EDTA	—	—	—	—	—	10
MgCl ₂	5	5	5	5	5	—
ATP	5	5	5	5	—	—
Imidazole	20	20	20	20	20	20
pH 7.0						

In some experiments a rephosphorylating system (10 mM phosphocreatine + 1 mg ml⁻¹ creatine kinase) was added in the activating, preactivating and relaxing solutions and the [KCl] was reduced to 40 mM. The results in the two sets of solutions were similar. Numbers denote concentrations in mM.

the surface membrane from the fibres by immersion for about 30 min in a relaxing solution containing 0.5% of a nonionic detergent, Brij 35, and then transferred to a relaxing solution. Two or more activation-relaxation cycles were performed (Fig. 1a). The regulatory light chains were removed by transferring the bundle to rigor solution, washing in a similar solution containing no added Mg²⁺ and leaving the bundle in desensitising (EDTA) solution for 15–30 min. The bundle was washed in rigor solution and transferred to relaxing solution. Tension rose slowly over a period of a minute or two. (A record from a second fibre bundle is shown in Fig. 1e and is to be contrasted with the relaxation from rigor in a similar procedure before desensitisation, shown in Fig. 1d.) When the fibre bundle was transferred from relaxing to activating solution, little extra tension developed (Fig. 1b), showing that desensitisation was almost complete. Resensitisation was achieved by placing the bundle for 30 min in a rigor solution containing 1 mg ml⁻¹ scallop regulatory light chains⁴. Figure 1c shows that calcium control was essentially completely reestablished.

The tension generated in the desensitised and resensitised states is due to an active turnover of cross-bridges similar to that before EDTA treatment. This was shown by apply-

ing a hand-operated release of about 1% of the fibre bundle length during a contraction to obtain a crude assessment of the ability of the bundle to redevelop tension. Results from such an experiment are shown in Fig. 1f–j. (This fibre bundle showed complete desensitisation, but only partial resensitisation was achieved, that is, some active tension developed in relaxing solution; compare Fig. 1j and 1c.) In rigor solution at any stage of the procedure there was little tension recovery after a release, shown in Fig. 1i for the fibre bundle after desensitisation. By contrast, recovery of tension after a release occurred in contractions in activating solution before EDTA treatment (Fig. 1f) in both relaxing and activating solution after desensitisation (Fig. 1g and 1h, respectively), and in activating solution after resensitisation (Fig. 1j). The rate of recovery after a release was slower in the desensitised state than before treatment or after resensitisation, but this may have been due to depletion of ATP in the bundle because of the longer time it had contracted before the releases were carried out.

The tension developed by desensitised fibre bundles was invariably lower than the active tension before treatment, and there was a small amount of recovery of active tension when the bundles were resensitised. This may indicate that

Fig. 1 Tension records from chemically skinned muscle fibre bundles from the striated adductor of *Placopecten magellanicus*. Lower lines are tension baselines (because of drift in the RCA 5734 transducer used, these are in some cases approximate). A simple rapid activation technique⁵ was used in which a fibre bundle was first placed in a relaxing solution while a low EGTA concentration ('pre-activating' solution, Table 1) to allow a large buffered Ca²⁺ gradient when the bundle was transferred to an activating solution. Rapid relaxation was achieved using a high EGTA concentration ('quick relaxing' solution, Table 1). Symbols for solutions: PA, pre-activating; QR, quick relaxing; R, relaxing; Rig., rigor. The tension and time scales in *a* apply to all the records. Temperature 20–25 °C. First fibre bundle, *a*–*c*; *a*, activation-relaxation procedure before desensitisation; *b*, after desensitisation, the tension developed in relaxing solution is not much enhanced in activating solution; *c*, after resensitisation, the activation-relaxation response is similar to that of *a*. Solutions were as in Table 1 with no rephosphorylating system. This fibre bundle was not analysed for light chain content. Second fibre bundle, *d*–*j*; relaxation of tension when freshly skinned fibre bundle in rigor is placed in relaxing solution; *d*, development of tension when desensitised bundle in rigor is placed in relaxing solution; *e*, development of tension when desensitised bundle in rigor is placed in activating solution; *f*–*j*, tension responses to hand-operated releases of ~1% (at arrows); *f*, untreated bundle during activation; *g*, desensitised, in relaxing solution; *h*, desensitised, in activating solution; *i*, desensitised, in rigor solution; *j*, resensitised, during activation-relaxation procedure (the faster rise of tension towards the end of the contraction resulted from slowly restretching the bundle to its initial length). Solutions were as in Table 1 except that a rephosphorylating system was used in the pre-activating, activating and relaxing solutions. After a further period of exposure to regulatory light chains, which slightly improved the resensitisation, this fibre bundle was analysed for light chain content (Table 2d).

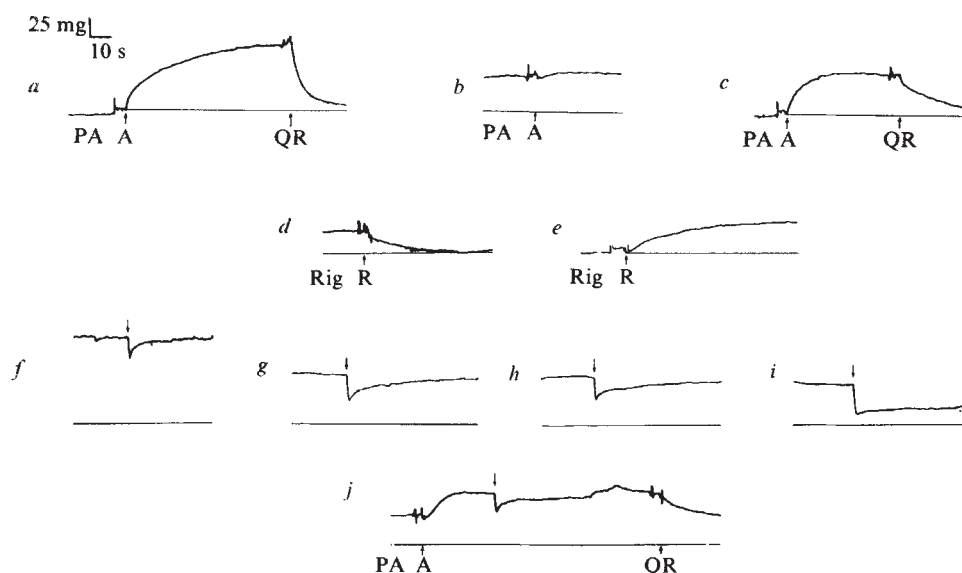


Table 2 Light chain content and calcium sensitivity of fibre bundles

Fibre bundle	Treatment	Light chain ratio	Ratio of tensions in relaxing/activating solution	ATPase activity in Ca^{2+} ($\mu\text{mol}^{-1} \text{min}^{-1} \text{mg}^{-1}$)	Ratio of ATPase in EGTA/ Ca^{2+}
a	Control	0.88	0		
b	Control	1.04	0		
c	Desensitised	0.35	1.0		
d	Resensitised	0.87	0.4		
e	Resensitised	0.97	0.3		
f	Control, pooled	0.95	0	1.3	0.02
g	Desensitised, pooled	0.35	1.0	0.6	0.81
h	Resensitised, pooled	0.82		0.91	0.12
	homogenised				

The regulatory light chain content is obtained from the ratio of the regulatory light chains to the SH-light chains, as the procedures used do not remove the SH-light chains⁴. A ratio of 1 indicates that both myosin heads retained their regulatory light chains, the loss of one of the two regulatory light chains results in a complete desensitisation of ATPase activity^{2,4}. *a-e*, Measurements on individual fibre bundles used in mechanical studies. *f-h*, Pooled, homogenised fibre bundles, 2 independent measurements averaged. Pooled bundles were homogenised in 5 ml 50 mM KCl, 25 mM imidazole, pH 7.0, 1 mM MgCl_2 , 0.1 mM EDTA in the microattachment of a Sorvall Omnimixer for 5 s twice at 0°C. The suspension was centrifuged and resuspended in 1 ml 50 mM KCl, 25 mM imidazole, pH 7.0, 1 mM MgCl_2 . Aliquots were used for urea gel electrophoresis, protein determination and ATPase activity. ATPase was measured in a pH-stat in 10 ml 20 mM KCl, 1 mM MgCl_2 , 0.75 mM ATP and 0.1 mM EGTA at pH 7.5 to obtain rates in the absence of calcium. The rates with calcium were measured in a similar solution containing in addition 0.2 mM CaCl_2 .

removal of the regulatory light chain affects the properties of cross-bridges, but this point will require further investigation. Judged by the ratio of tension in relaxing solution to that in activating solution, the EDTA-treated fibres were 80–100% desensitised (five fibre bundles). By the same criterion, resensitisation was 60–100% complete (three fibre bundles).

Direct evidence for light chain content at the different stages of the procedure comes from urea gel analyses carried out on two control bundles that were activated once without any further treatment (*a,b*), on one bundle shown to be mechanically desensitised (*c*), and on two (partially) resensitised bundles (*d,e*). These results (Table 2) show a good correlation between light chain content and the mechanical results. The untreated controls have their full complement of regulatory light chains, about 65% of the regulatory light chains (1.3 per myosin compared with 1.0 per myosin in refs 2 and 4) were lost from the desensitised bundle, and the resensitised bundles have a light chain content similar to those of the control preparations. The degree of mechanical resensitisation was less than would be anticipated from the light chain content, but this may have been due to the fact that the fibres were treated with EDTA at room temperature, possibly resulting in some irreversible loss of calcium control in actomyosin. It is noteworthy that, in agreement with previous studies⁴, only stoichiometric amounts of regulatory light chain are bound when combined with desensitised bundles, although light chain was added in great excess. The ATPase activity and the regulatory light chain content of pooled bundles obtained in parallel with mechanical experiments also show an excellent correlation with the mechanical studies with respect to the presence or absence of calcium control, according to the treatment used (Table 2).

Although these results are preliminary because of the few days available to us for the mechanical experiments, the striking agreement between the mechanical and biochemical experiments suggests that we have succeeded in moving and recombining regulatory light chains with fibre bundles of sufficient size for detailed mechanical measurements. The results indicate a good correlation between the control by calcium of ATPase activity and of tension. Finally these studies show more directly than has hitherto been possible that calcium regulation of contraction in scallop striated muscle is associated with myosin.

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Changes in crossbridge attachment in a myosin-regulated muscle

THE accompanying paper by Simmons and Szent-Györgyi¹ presents a direct confirmation that regulation of activity in scallop muscles is mediated by the thick filaments: when one particular polypeptide (a regulatory light chain) is removed from the myosin molecules, tension development is no longer inhibited by the absence of calcium ions. Yet we still have no picture of the structural events associated with myosin-linked regulation, comparable with (for example) the 'tropomyosin-shift' model for the interactions of calcium, troponin and tropomyosin in actin-linked regulation (see, for example, Wakabayashi *et al.*²). We report here that removal of the regulatory light chain can lead to a structural change observed by X-ray diffraction. Our observations on the scallop may have a more general significance since myosin-linked regulation is found, in addition to actin-linked regulation, in many types of muscle³.

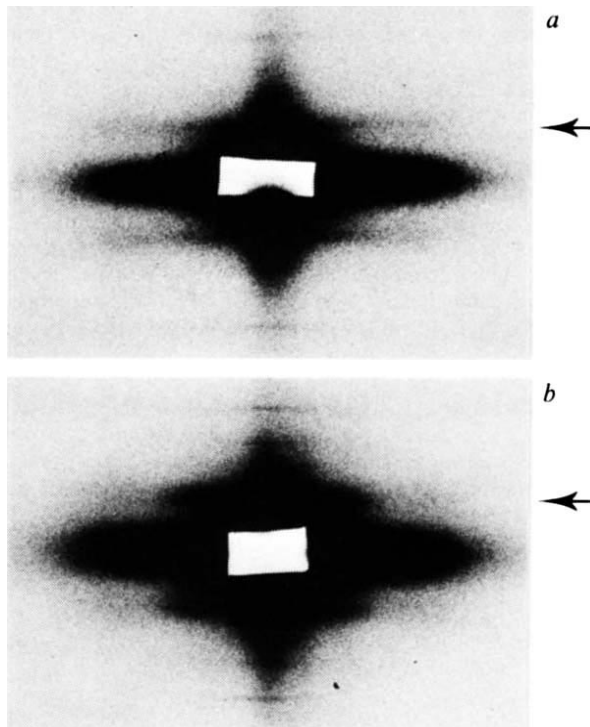


Fig. 1 X-ray diffraction patterns from scallop striated muscle. *a*, Rigor muscle; *b*, desensitised muscle. The weak 145-Å meridional reflection is unchanged, but there is a $\sim$ threefold increase in the intensity of the inner part of the 385-Å layer line (arrow), and a smaller increase on and near the meridian on the 193-Å layer line. The specimens were prepared either by glycerination (equal volumes of glycerol and rigor buffer) or by detergent skinning. Rigor buffer contained 40 mM KCl, 8 mM $MgCl_2$, 1 mM EGTA, 10 mM histidine, 0.2 mM sulfadiazine, 3 mM NaN_3 , pH 7.0; skinning solutions contained in addition 1–5 mM ATP and either 1% Triton X-100 or 0.5% Brij 35. Detergent-skinned preparations relaxed at least partially in ATP-containing solutions after 1–4 h in skinning solution, whereas the response of glycerinated muscles to ATP was less predictable. The orientation of specimens was in general better for glycerinated muscles. Thin bundles of fibres were mounted in a cell in the appropriate bathing solution, and X-ray diagrams recorded on Ilford Industrial G film using a mirror-monochromator camera with 400 mm specimen-to-film distance. The imperfect orientation and relatively poor order in rigor and desensitised muscles necessitated rather long exposure times, 24 h on an Elliott GX6 rotating anode generator at 900 W.

The scallop muscle is as well suited to structural as to biochemical studies. The very detailed and unusual X-ray pattern from the myosin filaments of the relaxed muscle has been described^{1,3}. Notable features of the crossbridge surface lattice are the non-equivalence of adjacent 145-Å levels, and the apparently unusually small azimuthal separation of crossbridges. Unfortunately, it is not yet feasible to observe the structural change when this relaxed crossbridge array is activated (either by calcium or by removal of light chains); unless the muscle has been stretched to the point of zero filament overlap, the bridges are continuously cycling through several attached and unattached states. In rigor, however, bridges are in a long-lasting state of attachment (possibly corresponding to that at the end of the power stroke in the contracting muscle), so that any difference resulting from the absence of the regulatory light chain should be observable.

Specimens of the sea scallop *Placopecten magellanicus* were obtained from the Marine Biological Laboratory, Woods Hole. After removal of the viscera, the smooth 'catch' portion of the adductor muscle was cut, and the striated adductor muscle allowed to stretch under the

tension supplied by the hinge ligament. The resulting sarcomere lengths, measured by laser diffraction, were usually in the range 2.4–2.8 μ m, corresponding to about two-thirds of maximum overlap of thick and thin filaments⁴.

The small-angle X-ray pattern from rigor scallop muscle (Fig. 1) shows strong off-meridional diffraction on layer lines that arise from myosin crossbridges attached to actin. These layer lines index on the actin repeat of 770 Å, with even orders dominant at small angles and odd orders at wider angles. On the low-order layer lines, the peaks lie at a small and constant radius (about $R = 0.005 \text{ Å}^{-1}$) from the meridian. Sampling due to the hexagonal lattice of thick filaments is restricted to the equator (Fig. 2), as expected from the limited order in the muscle⁵. Very weak meridional reflections occur at 385, 193 and 128 Å, similar to those seen in patterns from relaxed muscles^{1,3}. No effect of calcium ions on the low-angle pattern of the rigor muscle was observed.

When the light chain was removed from rigor muscle by treatment with EDTA (Table 1), there was a striking increase in intensity on the near-meridional part of the 385-Å layer line (out to about $R = 0.005 \text{ Å}^{-1}$) (Fig. 1*b*). The outer part of the layer line does not seem to change its profile or intensity. A similar intensity increase is observed on the

Table 1 Light chain content and calcium sensitivity of muscle preparations

Muscle preparation	Treatment	Light chain ratio	Ratio of ATPase in EGTA/ Ca^{2+}
1	Rigor	1.07	0.12
	EDTA	0.48	0.78
2	Rigor	0.98	0.33
	EDTA	0.57	0.75
3	Rigor	0.96	—
	EDTA	0.53	0.32
	Resensitised	0.76	0.07
4	Rigor	0.96	—
	EDTA	0.56	0.48
	Resensitised	0.93	0.22
5	Rigor	0.96	—
	EDTA	0.50	0.55
	Resensitised	1.10	0.23
Range and average for a larger number of preparations	Rigor ($n = 16$)	0.84–1.07 (0.94)*	0.10–0.35 (0.24)
	EDTA ($n = 14$)	0.30–0.62 (0.47)	0.32–0.92 (0.72)
	Resensitised ($n = 9$)	0.54–1.10 (0.76)	0.07–0.77 (0.46)

Thin bundles of glycerinated muscle, 0.3–1 mm in diameter, were used for X-ray diffraction experiments. Several such bundles were treated in parallel to provide enough material for diffraction, ATPase measurements and urea-acrylamide gel electrophoresis. Washing for 4–6 h at 4 °C in 10 mM EDTA, 40 mM NaCl, 25 mM Tris-HCl, pH 8, resulted in stoichiometric removal of one regulatory light chain per myosin. EDTA was washed out of the bundles with 40 mM NaCl, 25 mM Tris, pH 8; samples were then exposed to X rays in rigor buffer. For resensitisation, bundles were incubated with 0.1–1 mg ml⁻¹ scallop regulatory light chain in rigor buffer for 6 h at 4 °C, with gentle shaking. The accompanying paper¹ describes the definition and measurement of light-chain content and calcium sensitivity of ATPase activity. In the best experiments, EDTA treatment removed one regulatory light chain per myosin, and the ATPase activity showed substantially less dependence on the presence or absence of calcium. Resensitised muscles contained a full complement of light chains, and the calcium sensitivity was restored (preparations 1–5). We found significant variations in the extent of desensitisation and resensitisation, no doubt due to the thickness of the muscle bundles used. The range of values for a larger number of preparations is indicated.

*Average values shown in parentheses.

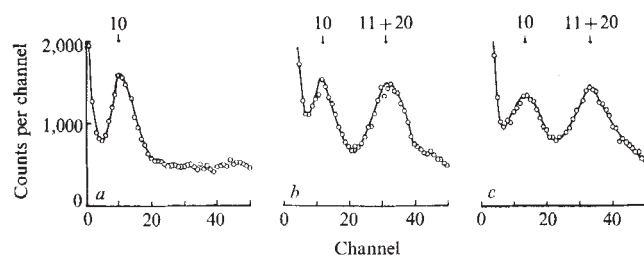


Fig. 2 Equatorial diffraction from scallop muscle recorded on a one-dimensional position-sensitive detector, designed by W. Phillips. A wedge-shaped aperture in front of the detector reduces the central scatter and improves the signal-to-noise ratio at higher angles, but changes the relative peak heights of the reflections. Disorder in the hexagonal lattice of thick filaments causes broadening and overlap of reflections beyond (10). Nevertheless, the relative intensities change substantially between *a*, living relaxed and *b*, rigor muscle, probably reflecting a transfer of mass (crossbridges) from thick to thin filaments. Desensitised muscle (*c*) is little different from rigor, suggesting that no further mass transfer occurs when the regulatory light chain is removed.

193-Å layer line. There are no marked intensity changes in the rest of the pattern, including the equatorial peaks (Fig. 2). Muscles desensitised by removal of light chain re-bind regulatory light chains stoichiometrically and regain calcium sensitivity (Table 1); in the best experiments the X-ray patterns then become indistinguishable from those of rigor muscle, although both the degree of resensitisation and the reversal of the X-ray intensities were somewhat variable.

Electron microscopy has so far failed to reveal any structural changes accompanying desensitisation. Sectioned muscles show no changes in either the period or the orientation of the crossbridges attached to the thin filaments, and negatively stained actin filaments with scallop heavy meromyosin attached show similar arrowhead configurations whether or not the light chain is present.

The geometry of interaction of actin and myosin can be pictured rather precisely if the structure and arrangement of the filaments are known (as illustrated by recent studies of insect⁶ and crustacean⁷ muscles). We do not yet know the actin pitch in scallop muscle, but note that the diffraction from actin is typical of that found for other muscles and, as expected⁸, shows none of the strong diffraction from troponin⁷. The weak meridional orders of 385 Å presumably arise from 'head-to-tail' overlaps between tropomyosin molecules. It may well be that the actin filaments are randomly orientated in the rather irregular lattice; but despite variations in lattice order, many invertebrate muscles (such as insect⁶ and *Limulus*⁹) show, as the scallop does, that the 385 Å and 193 Å layer lines run very close to the meridian. We can take the general conclusion from the more precise analysis of crustacean and insect muscle rigor patterns that this effect results from a tendency for crossbridges to attach preferentially at certain points along the actin filaments, with a repeat close to the actin pitch. The rigor pattern poses the question of how myosin 'crowns' are matched to the surrounding 'target areas' on the thin filaments¹⁰. In general, we must expect that not all the S1 heads will attach to actin, and that the behaviour of those which remain unattached will be a significant factor influencing diffracted intensities. In particular, the tendency (or otherwise) for one head to be influenced by the other head of the same myosin molecule must be considered. The X-ray change we have observed shows that removal of one regulatory light chain changes reversibly the distribution of myosin crossbridge density associated with actin. The equator of the pattern em-

phasises that this redistribution occurs with little change in the average proximity of crossbridges to actin. Fuller analysis of the scallop patterns will distinguish between change of number of attachments and form of attachments.

Biochemical studies of scallop muscles have shown, for the first time for any muscle, the importance of head-head interactions, indicating that inhibition of the bridges depends on the interactions between heads¹¹. (As in thin filament regulation, only nucleotide-containing myosin is inhibited from binding to actin by low calcium concentrations¹².) The relaxed X-ray pattern must in some way embody the particular cooperative structural relationship that corresponds to inhibition. The release of this inhibition by calcium is presumably due to a modification of the head-head interaction. In principle, we may hope to study this modified structural relationship by X-ray observations of contracting muscles. So far, we have looked only at another stable state in the crossbridge cycle, namely rigor. Here some of the bridges are 'frozen' into interactions with actin (and we know little of the stages by which this state is reached from the relaxed state). The structure attained on removal of the light chain from rigor muscle illustrates the possibility of changing the pattern of association of myosin with actin; changes in head-head affinity could explain both this change and that which is induced by calcium on activation. The myosin-linked regulation in molluscan muscles may be more specialised than that in muscles which are regulated also through actin. But all myosins are two-headed, and light chains from many myosins can substitute for the scallop light chain in regulating scallop myosin¹³. Head-head interactions may well be involved in the regulation of crossbridge dynamics in other muscles.

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Behaviour of myosin projections during the staircase phenomenon of heart muscle

PEAK tension of an excised heart muscle increases with the frequency of contraction¹. This 'staircase' phenomenon has been attributed to changes in the distribution of Ca ions; it has been proposed that the sarcoplasmic Ca²⁺ concentration increases with the frequency of contraction²⁻⁴. The increase in the sarcoplasmic Ca²⁺ is supposed to raise the peak tension by changing the behaviour of contractile proteins; however, no such molecular change has yet been clearly demonstrated. In the present study, we have investigated the behaviour of myosin projections during the staircase phenomenon by the X-ray diffraction method, in an attempt to clarify the underlying molecular events. The results have indicated that an increase in the contraction frequency causes an increase in the number of myosin projections transferred to the vicinity of the thin filaments during contraction.

A papillary muscle was isolated, together with the ventricular septum from the right ventricle of the canine heart. The muscle was cross circulated with arterial blood of a donor dog according to the method described by Endoh and Hashimoto⁵: the anterior septal artery was cannulated and connected to the carotid artery of the donor dog through a perfusion pump (Harvard model 1210). The perfusion pressure was maintained at 100 mmHg. The venous outflow from the preparation was returned to the jugular vein of the donor dog.

The ventricular septum was set in a Perspex chamber surrounded by a warm bath (38 °C), and the papillary muscle was pulled out of the chamber through a close-fitting hole. The base of the muscle was stitched to the edge of the hole⁶. The tip of the muscle was tied to an isometric tension transducer (Shinkoh, UL). A branch of the arterial tubing was used to drip arterial blood on the surface of the papillary muscle in order to prevent the surface from cooling down and drying out. The muscle was stimulated with square pulses of 5 ms duration through a punctate platinum electrode⁶. At the beginning of each X-ray exposure, the muscle length was adjusted to L_{max} , where tension production was maximal.

A low-angle X-ray camera of the mirror-monochromator type⁷ was used. A powerful source of X rays was supplied by a rotating-anode generator (Rigaku, FR) operated at 50 kV with a tube current of 70 mA (focal size 1 × 0.1 mm); such a high power density was made possible by using an anode of large diameter (30 cm) rotating at high speed (9,000 r.p.m.). The papillary muscle was placed vertically, and the equatorial diffraction pattern was recorded on X-ray film (Sakura N) with a specimen-to-film distance of 45 cm. An electromagnetic shutter was placed between the monochromator and the muscle. The shutter was coupled with the tension transducer, and passed X rays only during the systolic phase; the shutter was opened as the tension exceeded two thirds of peak tension and closed as it fell below half of the peak tension⁸. Approximately 10,000 contractions were needed to obtain one equatorial pattern. As the preparation was capable of contracting more than 20,000 times with little decline of the peak tension, it was possible to record two patterns from each muscle. One of the two patterns was recorded at a contraction frequency of 120 min⁻¹ which we chose as the 'control' frequency, and the other pattern at either 60 or 180 min⁻¹. Each pattern was densitometered, and the integrated intensities of the 1,0 and the 1,1 reflections from the hexagonal array of the myofilaments were measured to obtain the intensity ratio ($I_{1,0}/I_{1,1}$) (for the method of measuring the intensities^{8,9}). Changes in the peak tension and the intensity ratio, caused

by altering the contraction frequency in each muscle, were expressed as percentages of the values given by the same muscle at the control frequency.

At the control frequency, the peak systolic tension was 287 ± 26 g cm⁻² (mean $\pm$ s.e.m., $n=10$) and the intensity ratio was 1.34 ± 0.07 ($n=10$). When the frequency was increased to 180 min⁻¹, the peak tension increased by $26 \pm 2.7\%$ ($n=5$) and the intensity ratio decreased by $19 \pm 4.5\%$ ($n=5$). When the frequency was decreased from the control to 60 min⁻¹, the peak tension decreased by $26 \pm 5.8\%$ ($n=5$) and the intensity ratio increased by $30 \pm 8.0\%$ ($n=5$). All these changes in the peak tension and the intensity ratio were statistically significant ($P < 0.05$).

Changes in the intensity ratio have been attributed to radial transfer of myosin projections from the vicinity of the thick filaments to that of the thin filaments^{10,11}; a decrease in the intensity ratio indicates an increase in the number of projections in the vicinity of the thin filaments. Therefore, the present results suggest that the rise of the peak tension caused by increasing the frequency of contraction is accompanied by an increase in the number of myosin projections associated with the thin filaments during the systolic phase.

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A temperature-sensitive yeast mitochondrial mutant with altered cytochrome c oxidase subunit

SOME of the subunits of yeast cytochrome oxidase, cytochrome *b* and ATPase are synthesised on mitochondrial ribosomes. Inhibition of protein synthesis by antibiotics that specifically affect mitochondrial ribosomes leads to the absence of three subunits of cytochrome oxidase, at least three subunits of ATPase, and a 30,000 molecular weight polypeptide believed to be cytochrome *b* (ref. 1). This indicated that the mitochondrion is the site of synthesis of these polypeptides, but only inferences could be drawn as to whether their mRNAs were transcribed from mitochondrial DNA (mtDNA) or from nuclear DNA. We report here the isolation of a temperature-sensitive mutant of *Saccharomyces cerevisiae* which is mitochondrially inherited and has an altered subunit of cytochrome *c* oxidase. The simplest interpretation of our results is that mtDNA codes for this subunit.

The isolation of MnCl₂-induced mitochondrial mutations specifically affecting the synthesis of cytochrome oxidase, cytochrome *b* and ATPase has led to the possibility of directly

determining a gene-protein relationship for mitochondrial genes². These mutations most frequently lead to a loss of cytochrome oxidase activity, and a majority of such mutations map at the *oxi 3* locus. Such mutations might be expected to affect the primary structure of one of the subunits of cytochrome oxidase, but unfortunately almost all known mutations of this region lead to a loss of detectable subunit I (ref. 3).

In the hope of obtaining mutations leading to detectable alterations in the primary structure of a mitochondrial translation product, we isolated conditional temperature sensitive (*ts*) mutations and characterised cells grown at permissive and nonpermissive temperatures. A haploid strain of *S. cerevisiae*, BG-9A (α *ino* 1-13 *ino* 4-8 *ade* 2 (E^R)) was subjected to $MnCl_2$ mutagenesis⁴, followed by the inositol selection scheme of Henry *et al.*⁵ (E.M.S. *et al.*, in preparation). Mutant strains were selected on YPGE plates (1% Difco yeast extract, 2% Difco peptone, 3% (v/v) glycerol, 2% (v/v) ethanol, 2% agar) for ability to grow at the permissive temperature (25 °C) but not at the nonpermissive temperature (36 °C). One such mutant strain was designated IM 9-4.

The location of the mutation in IM 9-4 was determined to be mitochondrial by the following criteria. When the mutant IM 9-4 (ρ^+) was crossed with a wild-type respiratory-sufficient haploid strain, the resultant diploids showed mitotic segregation of the temperature sensitive (*ts*) phenotype on YPGE plates. Sporulation of these diploids yielded spores which segregated 4:0 or 0:4 (wild type:*ts* mutant), depending on the phenotype of the diploid. When IM 9-4 (ρ^+) was crossed with a ρ^0 tester strain, D585 11C EB, all the resulting diploids had the mutant *ts* phenotype. In the reciprocal cross of IM 9-4 (ρ^0), obtained after ethidium bromide treatment of IM 9-4 (ρ^+), and wild type D585 11C (ρ^+), only wild-type diploids, with respect to growth on YPGE plates, were generated.

Fig. 1 Cytochrome spectra of wild-type and mutant diploid cells grown at 25 °C and 36 °C. BG-9A diploid and IM 9-4 diploid strains were grown to early stationary phase on a medium containing 1% Difco yeast extract, 2% Difco peptone and 1% galactose. The cells were washed with deionised water, suspended in cold MTE (0.6 M mannitol, 20 mM Tris-acetate, pH 7.5, 2 mM EDTA) and homogenised in a Braun homogeniser for 45 s using liquid CO_2 for cooling. Cellular debris was removed by two low-speed centrifugations (1,475g for 10 min) and the mitochondria pelleted from the supernatant by centrifugation at 12,000g for 20 min. The mitochondria were suspended in a small volume of MTE and the suspension made 2% with respect to cholic acid. Difference spectra were determined at room temperature by reading sodium dithionite-reduced samples against potassium ferricyanide-oxidised samples on an Aminco double-beam spectrophotometer. *a*, The difference spectra for the wild-type diploid strain grown at 25 °C (upper trace) and grown at 36 °C (lower trace). *b*, The difference spectra for the mutant diploid strain grown at 25 °C (upper trace) and grown at 36 °C (lower trace).

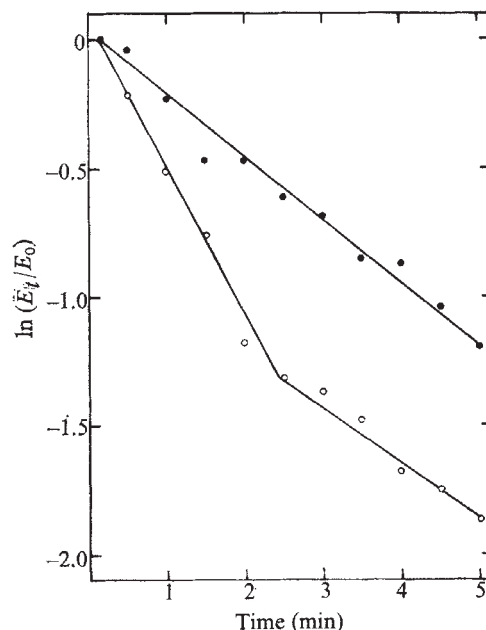
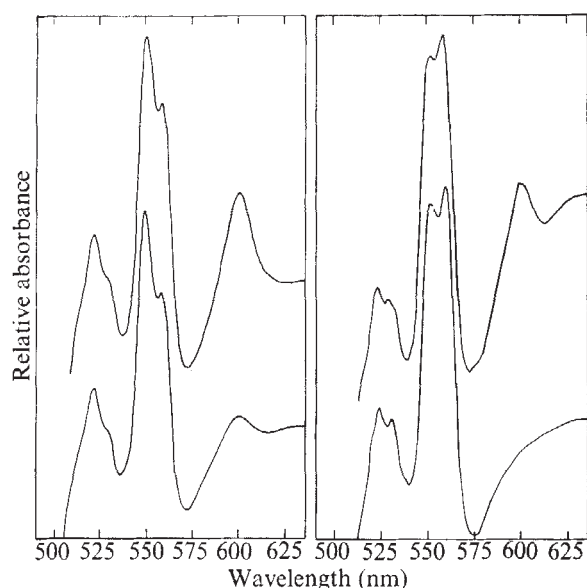


Fig. 2 Heat inactivation of cytochrome *c* oxidase activity from wild-type and mutant diploid strains. BG-9A diploid and IM 9-4 strains were grown in YP Gal medium at 25 °C to late logarithmic phase, washed with deionised water and the mitochondria prepared as described in Fig. 1 legend. The mitochondria were suspended in MTE buffer and an aliquot of the mitochondrial suspension was added to MTE buffer preheated to 50 °C. At various times, aliquots were withdrawn, added to an equal volume of chilled MTE buffer at 0 °C, and assayed for cytochrome *c* oxidase activity as described in Table 1 legend. The enzyme activity of the cytochrome *c* oxidase time = 0 was E_0 and at time = t , E_t , where t represents the time for which the mitochondrial suspension was exposed to 50 °C.

The parent strain, BG-9A, from which the mutant IM 9-4 was derived, is resistant to erythromycin (*E*), and sensitive to chloramphenicol (*C*), oligomycin (*O*) and paramomycin (*P*). When IM 9-4 (ρ^+ $C^S E^R O^S P^S$) was crossed with strain 290-6A (ρ^+ $C^R E^S O^R P^R$), the *ts* marker showed the same transmission frequency as the C^S , E^R , O^S and P^S markers. This would be expected if the *ts* marker is located on the same molecule of mDNA as the antibiotic markers.

Nonconditional mitochondrial mutants with the same phenotype that IM 9-4 displays at the restrictive temperature have been shown to map at several regions of the genome³. These regions of the mitochondrial genome—*oxi 1*, *oxi 2* and *oxi 3*—have in turn been defined by a series of mutants having specific deletions of mitochondrial DNA. Crosses of some of these deletion mutants with mutant IM 9-4 show that the latter maps in the region defined by deletion 10-150, in which a large portion of the *oxi 3* region has been deleted⁴. In addition, deletion of the mitochondrial *ts* locus as a result of $MnCl_2$ treatment was correlated with the deletion of known mutations at the *oxi 3* locus but independent of mutations at both *oxi 1* and *oxi 2*. Independence of the *ts* mutation in IM 9-4 from the loci *oxi 1* (defined by mutant 9-94, ref. 3) and *oxi 2* (defined by mutant 9-3, ref. 3), was confirmed by recombinational analysis (R.B.N. *et al.*, in preparation). The data thus indicate that strain IM 9-4 carries a *ts* mutation mapping in the *oxi 3* region of mitochondrial DNA.

To facilitate the study of the biochemical effect of this mutation, diploid strains, able to grow using galactose as a carbon source, were generated as neither the haploid parent strain (BG-9A) nor the *ts* mutant strain derived from it grew well on galactose-containing media. The diploids used resulted from mating each strain to an ethidium bromide derivative of galactose-utilising strain J5 (α , *ade* 5, *lys*, *GAL*).

The mutant diploid, IM 9-4 $\times$ J5 (ρ^0) (hereafter referred to as the IM 9-4 diploid) grew with a doubling time of 5.7 h on YPGE medium at 25 °C whereas the wild-type diploid, BG-9A $\times$ J5 (ρ^0) (the BG-9A diploid) doubled every 2.5 h in the same conditions.

When the mutant diploid was grown in YPGE medium at 25 °C and then shifted to 36 °C, growth stopped after about two generations at the nonpermissive temperature. This growth inhibition was reversible when the culture was returned to the permissive temperature.

When mitochondria were isolated from mutant diploid cells grown at 25 °C or 36 °C in a medium containing 1% Difco yeast extract, 2% Difco peptone and 1% galactose and examined for their cytochrome content, cytochromes *a* + *a3* were missing from mutant mitochondria isolated from cells grown at 36 °C but were present in reduced amounts in mutant mitochondria obtained from cells grown at 25 °C (Fig. 1).

When mitochondria were assayed for respiratory enzymes, cytochrome *c* oxidase activity was depressed in mutant diploid cells grown at 36 °C on a medium containing 1% Difco yeast extract, 2% Difco peptone and 2% galactose (YP Gal) (Table 1). Thus the loss of cytochromes *a* + *a3* is correlated with a loss of cytochrome *c* oxidase activity when the mutant is grown at 36 °C.

To determine the thermal lability of the cytochrome *c* oxidase of the mutant strain, mitochondria were isolated from cells grown at 25 °C on YP Gal medium and exposed to 50 °C for various periods. The cytochrome *c* oxidase activity was found to be more heat labile than the same activity from wild type cells (Fig. 2). In contrast, the heat stability of mutant NADH-cytochrome *c* reductase is comparable with that of wild type (data not shown). Thus, the heat lability is not a general membrane effect. When mitochondria from mutant cells grown at 25 °C were assayed for cytochrome *c* oxidase at 25 °C, 30 °C and 35 °C no difference in activity, relative to the activity of wild type cells, was observed. Thus cytochrome *c* oxidase from the mutant strain, assembled at 25 °C retains activity at 35 °C comparable with that of the wild type enzyme. Yet, the cytochrome *c* oxidase of mitochondria from cells grown for four to five generations at 36 °C had less than 1%

Table 1 Specific activities of respiratory-chain enzymes in wild-type and mutant diploid strains

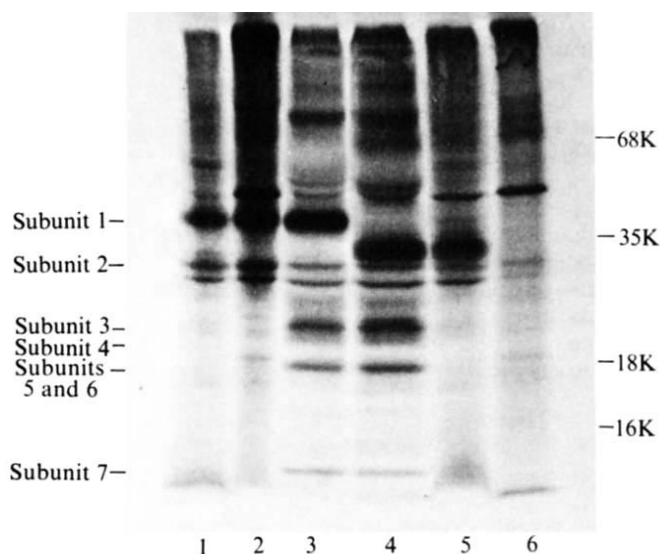
Strain	Growth temperature (°C)	Cytochrome <i>c</i> oxidase	Specific activity NADH-cytochrome <i>c</i> reductase	Succinate-cytochrome <i>c</i> reductase
BG-9A × J5 (ρ^0)	25	1.0	0.31	0.08
	36	1.0	0.10	0.15
IM 9-4 × J5 (ρ^0)	25	0.51	0.62	0.07
	36	0.005	0.46	0.09

Mitochondria were isolated (see Fig. 1 legend) from wild-type and mutant diploid cells grown to late logarithmic phase on YP Gal medium at 25 °C and 36 °C. The mitochondrial pellets were suspended in MTE buffer and the suspensions assayed for cytochrome *c* oxidase, NADH-cytochrome *c* reductase and succinate-cytochrome *c* reductase according to the procedure of Tzagoloff *et al.*⁶. The specific activities are expressed as μmol of substrate oxidised or reduced per min per mg of protein. Protein was determined using the procedure of Lowry *et al.*⁷.

of the activity of cytochrome *c* oxidase found in wild type mitochondria and lacked cytochrome *a* + *a3*. If cytochrome oxidase previously formed at 25 °C was heat labile *in vitro* at 36 °C one would expect an immediate decrease in oxygen consumption after the shift. However, the oxygen consumption of mutant cells grown on YP Gal medium shifted from 25 °C to 36 °C continues to increase at a rate proportional to cell growth for about 1.5 generations, after which time the oxygen consumption decreases (data not shown).

The loss of cytochrome *c* oxidase activity could be due to a long-term instability of the enzyme at 36 °C and/or could reflect an inability of newly synthesised altered subunits to be assembled into the enzyme complex. The former suggestion could indicate

Fig. 3 Separation of mitochondrial translation products and cytochrome *c* oxidase immunoprecipitates from wild-type and mutant diploid cells using SDS-urea polyacrylamide gel electrophoresis. Mitochondrial translation products were labelled with $^{35}\text{S}\text{O}_4^{2-}$ according to the procedure of Douglas and Butow⁹ with the modifications noted below. The BG-9A diploid and IM 9-4 diploid strains were grown to a cell density of approximately $4 \times 10^7 \text{ ml}^{-1}$ in 10 ml YP Gal medium at 25 °C. The cells were washed twice with sterile water and suspended in 3 ml low-sulphate medium supplemented with adenine ($20 \mu\text{g ml}^{-1}$), non-sulphur-containing amino acids ($50 \mu\text{g ml}^{-1}$) and 2% galactose. The cells were incubated with shaking for 30 min at 25 °C at which time 200 μCi carrier-free ^{35}S -sulphuric acid (NEX-042, from New England Nuclear) was added. Five minutes before the addition of the label, cycloheximide was added to a concentration of $125 \mu\text{g ml}^{-1}$. The cells were labelled for 70 min and then 3 ml of chase solution (0.2% Na_2SO_4 , 1% casamino acids) were added. After 15 min the cells were pelleted by centrifugation, washed twice with 5 ml of the same chase solution and frozen at -20 °C. Mitochondria were isolated as described in Fig. 1 legend, except that the MTE buffer contained butylated hydroxyphenol ($50 \mu\text{g ml}^{-1}$) and homogenisation in the Braun homogeniser was for 4 min using the apparatus designed by Needleman and Tzagoloff¹⁰. The mitochondrial pellets were suspended and lysed in a solution containing 20 mM Tris-HCl, pH 6.8, 2 mM EDTA, 2% SDS (Pierce), 10 mM DTT and 10% glycerol. The lysate was then heated at 100 °C for 8 min. Immunoprecipitated cytochrome *c* oxidase was obtained from the wild-type and mutant diploid cells using the following protocol. Thirty ml of cells were grown to a cell density of $5 \times 10^7 \text{ ml}^{-1}$ in YPGE medium at 25 °C. The cells were washed twice with sterile water and suspended in 10 ml low-sulphate medium supplemented with adenine ($20 \mu\text{g ml}^{-1}$), non-sulphur-containing amino acids ($50 \mu\text{g ml}^{-1}$), glycerol (3% v/v) and ethanol (2% v/v). After preincubation for 30 min at 25 °C, 800 μCi carrier-free ^{35}S -sulphuric acid was added and incubation continued for a further 80 min. The cells were then washed with chase solution as above, suspended in MTE buffer containing 1 mM DTT and the mitochondria isolated as described in Fig. 1 legend. The mitochondria were suspended in MTE buffer at 3–6 mg protein ml^{-1} . Solubilised cytochrome *c* oxidase was obtained by adding KCl (final concentration 1 M) and potassium deoxycholate (final concentration, 0.5 mg per mg protein), stirring the suspension for 60 h at 4 °C, and then centrifuging at 100,000g for 60 min (ref. 11). The cytochrome *c* oxidase was immunoprecipitated from the supernatant using the double antibody technique¹². Cytochrome *c* oxidase antiserum was the gift of G. Schatz and goat anti-rabbit immunoglobulin was purchased from Miles. Immunoprecipitates were solubilised as described above for the mitochondrial pellets. Aliquots (containing approximately 30,000 c.p.m. of trichloroacetic acid-precipitable counts), adjusted to 8 M in urea, were applied to a 10–15% exponential polyacrylamide gradient gel containing 0.1% SDS, 8 M urea and the ethanolamine/triethanolamine buffer system of Wu and Bruening¹³. After electrophoresis, the gels were stained with Coomassie brilliant blue, dried and autoradiographed using Kodak No-screen (NS-5T) X-ray film. Lane 1, mitochondrial translation products from the BG-9A diploid strain, grown and labelled at 25 °C. Lane 2, mitochondrial translation products from the BG-9A diploid strain, grown and labelled at 36 °C. Lane 3, cytochrome *c* oxidase immunoprecipitate obtained from the BG-9A diploid strain. Lane 4, cytochrome *c* oxidase immunoprecipitate obtained from the IM 9-4 diploid strain. Lane 5, mitochondrial translation products from the IM 9-4 diploid strain, grown and labelled at 25 °C. Lane 6, mitochondrial translation products from the IM 9-4 diploid strain, grown for about 12 generations at 36 °C and labelled at 36 °C.



that subunit 1 is necessary for binding of the haem group as has been previously suggested⁸. In any event, the defect in mutant IM 9-4 is in the assembly of functional cytochrome oxidase at the nonpermissive temperature.

When the mitochondrial products of the BG-9A diploid and of the IM 9-4 diploid were specifically labelled in the presence of cycloheximide using $^{35}\text{SO}_4^{2-}$ and the polypeptides analysed by SDS-urea polyacrylamide gel electrophoresis, it was seen that one band from the mutant had a faster mobility than the corresponding band from wild-type cells (Fig. 3). From its molecular size and characteristic morphology this band was tentatively identified as subunit 1 of cytochrome *c* oxidase. The faster mobility of this hydrophobic subunit could reflect a polypeptide of an approximately 6,000 lower molecular weight, or be indicative of an altered conformation of a partially unfolded polypeptide. As the gel buffers are highly denaturing, the former explanation is more likely. Inclusion of the protease inhibitor phenylmethyl sulphonyl fluoride (2 mM) did not alter the gel pattern of wild type and mutant mitochondrial proteins.

Verification that the altered band and its wild-type counterpart corresponded to subunit 1 of cytochrome *c* oxidase was obtained by immunoprecipitating cytochrome *c* oxidase from mutant and wild-type cells. Cells were grown on YPGE medium at 25 °C, labelled using $^{35}\text{SO}_4^{2-}$ in the absence of cycloheximide to improve labelling of all mitochondrial proteins and the cytochrome *c* oxidase from disrupted mitochondria was immunoprecipitated with antiserum prepared against holoenzyme. When the cytochrome *c* oxidase immunoprecipitates from mutant and wild-type cells were compared using SDS-urea polyacrylamide gel electrophoresis, the cytochrome *c* oxidase from the mutant was indeed shown to have an altered subunit 1 (Fig. 3).

When the mitochondrial translation products of the mutant were labelled after the cells had grown for many generations (12–15) at 36 °C in YP Gal medium, subunit 1 was not detected. However, if the cells were grown at 36 °C for only four to five generations, the altered subunit 1 was still present, that is, the protein pattern is the same as for cells grown only at 25 °C. This probably indicates that the altered subunit is not stably integrated into the enzyme complex assembled at 36 °C. The unintegrated subunit is probably degraded.

Revertants of the haploid and diploid IM 9-4 strains have been obtained. These revertants, which regained their ability to grow on YPGE at 36 °C, have normal mitochondrial translation products (data not shown). The revertants so far isolated are all mitochondrial and could result from a back-mutation at the original lesion or from a second site suppressor, acting at the level of translation.

The data presented here are consistent with the hypothesis that the *oxi 3* locus encompasses the structural gene for subunit 1 of cytochrome oxidase, and more importantly, that yeast mRNA codes for at least one polypeptide—subunit 1 of cytochrome *c* oxidase.

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Evidence for 'splicing' of SV40 16S mRNA

By the application of a wide variety of restriction enzymes and of recently developed DNA sequencing techniques, large segments of the SV40 genome have now been sequenced^{1,2}. As a result, the expression of the viral genetic information can be studied in molecular detail. The major SV40-specific messenger RNA is a polyA-containing, cytoplasmic RNA which sediments at 16S and is only expressed after the onset of DNA replication³. *In vitro* translation studies have shown that it codes for VP1, the main structural protein of the virion (ref. 4 and A. Smith, personal communication). 16S mRNA hybridises mainly to the SV40 DNA *Hind* II+III restriction fragments K, F, J and G, which correspond to the region 0.95 to 0.17 on the standard map^{5,6}. The localisation of these restriction fragments and of 16S mRNA is illustrated in Fig. 1.

We have shown previously that the codon corresponding to the N-terminal alanine of the VP1 protein is located in *Hind* II+III fragment K at 15–17 nucleotides clockwise from the *Hind* II+III E/K junction^{7,8}. This pinpoints the beginning of the structural body of the VP1 gene. The termination codon of the VP1 gene, UGA, is located in *Hind* II+III fragment G at 78 nucleotides from the *Hind* II+III G/B junction⁹ and is followed by an untranslated 3'-terminal sequence. The ribonuclease-T1 oligonucleotide to which the polyA tail is attached actually spans the *Hind* II+III G/B junction at map position 0.17 (ref. 10). The 16S mRNA is capped^{11,12} and contains additional 6-methyl adenosine residues^{13,14}. We have now characterised the 16S mRNA in further detail by comparing its fingerprint with the nucleotide sequence of the DNA. From the results we can conclude that the 5'-leader sequence of the RNA is at least 180 nucleotides long and is derived from a separate region on the genome, located at 0.72 to 0.76 map units. The splicing of RNA segments transcribed from non-contiguous regions on the DNA has recently also been observed in other laboratories both for adenovirus mRNAs and SV40 mRNA^{15,16}. Our findings on the splicing of late SV40 mRNA were reported in brief previously¹.

SV40-specific late mRNA was labelled and isolated as follows: SV40-infected CV1 monkey cells (multiplicity of infection: 60 plaque-forming units (PFU) per cell) were labelled with ^{32}P -phosphate 24 h post-infection¹¹. After a further incubation period of 24 h at 37 °C, the cells were lysed, the nuclei were removed and the total cytoplasmic RNA was recovered by several successive phenol-chloroform extractions. PolyA-containing RNA was isolated on an oligo dT-cellulose column and fractionated according to size by preparative electrophoresis on a 1.4% agarose gel. SV40-specific RNA was selected out by hybridisation of each gel fraction onto Sepharose-bound SV40 DNA¹⁷. Alternatively, the hybridisation step preceded the preparative electrophoresis (see Fig. 2). The main virus-specific species had a mobility corresponding to 16S mRNA. It was further characterised by digestion with RNase T1 and fractionation of the resulting products by a two-dimensional

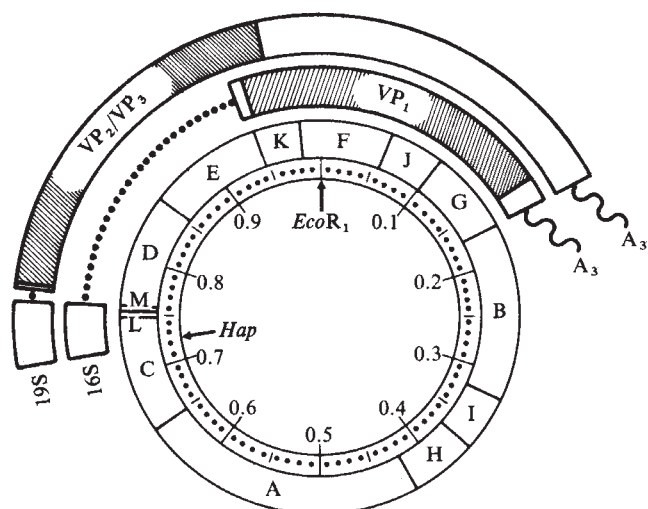


Fig. 1 *Hind* II+III restriction cleavage map of the SV40 genome and the localisation of the late 19S and 16S mRNA species. The single *Eco* RI restriction cleavage site is taken as reference point zero for the construction of the physical map. *Hind* II+III fragments are indicated by letters A through M. Striped blocks indicate the coding regions of the mRNAs, while clear blocks indicate the untranslated regions (that is, the 5'-leader and 3'-terminal segments); the wavy lines represent the 3'-terminal polyA tails. Dotted lines show the genome regions which are spliced away in the mRNA. This paper deals with the characterisation of the 16S mRNA, which codes for the major structural protein VP1 (the detailed characterisation of the late 19S mRNA will be documented elsewhere).

fingerprinting technique¹⁸. A typical pattern is shown in Fig. 3. All the oligonucleotides of chain length greater than four were eluted from the polyethyleneimine thin-layer plate and further identified by digestion with pancreatic RNase¹⁹. The resulting (partial) sequence of these T1-oligonucleotides was then compared with the actual DNA sequence of the late region of the SV40 genome.

All the T1-oligonucleotides predicted from the DNA sequence of the *Hind* II+III fragments K, F, J and G were indeed identified in the fingerprint (Table 1), with the exception of the oligonucleotide UUAACAACAACAAUUG, which spans the *Hind* II+III G/B junction. We have characterised a part of the aforementioned oligonucleotide as being linked to the polyA tail; it was present in a smeared spot at the origin which was analysed as UU(AAC)₃polyA. This location of the 3' end of the 16S mRNA on the SV40 DNA map confirms the assignment by Zain *et al.*¹⁰.

We then attempted to pinpoint the 5' end of the 16S mRNA. In the fingerprint (Fig. 3) only one unique (that is, present once per chain) T1-oligonucleotide, UUACUUCUG (spot No. 521), was clearly derived from the *Hind* II+III fragment E (ref. 20), while the other large, preceding ones were definitely missing. Their absence was not due to artefacts in the separation systems because these same oligonucleotides were clearly present in ribonuclease-T1 fingerprints of the larger, late 19S mRNA (manuscript in preparation). These data led to the conclusion that sequence representation in the 16S mRNA extends at most 40 nucleotides before the start codon of the VP1 protein (Fig. 4). However, it was not possible to locate the sequence AUU, which is directly linked to the cap at the 5' terminus^{1,11}, into this preceding segment. Moreover, after correlation of all the potential G-terminal DNA-oligonucleotides from the *Hind* II+III fragments E, K, F, J and G with actual T1 products of the 16S mRNA, a set of discrete T1-oligonucleotides remained unaccounted for,

Table 1 T1-oligonucleotide content of SV40 16S mRNA

Fingerprint spot no.*	C.p.m./nucleotide†	Calculated molarity‡	<i>Hind</i> II+III fragment§
004	374	1.2	K
013¶	622 or 2 × 311	1.0 × 2	L, J
014	287	0.9	F
016	220	0.7	F
023¶	990 or 4 × 248	0.8 × 4	K, K, G, G
024	316	1.0	G
026	209	0.7	M
034	274	0.9	J
037	235	0.8	L
044	276	0.9	J
057	230	0.7	K
104¶	927 or 3 × 309	1.0 × 3	K, F, J
112 + 103¶	1628 or 4 × 407	1.3 × 4	G, K, J, G
107	252	0.8	G
121¶	692 or 3 × 231	0.7 × 3	K, C, C
122¶	917 or 3 × 306	1.0 × 3	F, F, G
123¶	703 or 2 × 351	1.1 × 2	J, J
124	266	0.9	F
126	333	1.1	J
127 + 118¶	302	1.0	G, C
128	237	0.8	K
132	223	0.7	C
135	301	1.0	F
140	272	0.9	C
145	325	1.0	J
158	194	0.6	G
169	222	0.7	K
205	306	1.0	J
215	370	1.2	K
222	304	1.0	F
224 + 233¶	263	0.8	E-K, F
232	348	1.1	K
240	306	1.0	J
242	521 or 2 × 261	0.8 × 2	F, G
248 + 247 } ¶	239	0.8	F, F, F
2410 }			
303 + 312¶	2337 or 5 × 467	1.5 × 5	F, G, L-M, M-D, G
313	368	1.2	G
314 + 322¶	424	1.4	K, F, F
330	484	1.6	G
331	394	1.3	K
332	353	1.1	J
333	315	1.0	F
336 + 335¶	196	0.6	G, F
342	324	1.0	G
357	194	0.6	J
401 + 410¶	2188 or 5 × 438	1.4 × 5	F, G, C, J, C
412	324	1.0	C
413	283	0.9	F
420	581	1.9	J
426	224	0.7	G
502	364	1.2	J
504 + 522¶	465	1.5	F, F
520	303	1.0	C
521	309	1.0	E
525	297	1.0	F
611	946 or 2 × 473	1.5	G, G
654	281	0.9	G
846	269	0.9	K
878	218	0.7	G
938	310	1.0	G
1224**	297	1.0	F

* Numbers refer to the spots indicated in Fig. 3b. The code refers to the base composition, (see Fig. 3 and ref. 33). All spots corresponding to digestion products with chain length greater than four were cut out and counted by liquid scintillation.

† The c.p.m. per nucleotide is obtained by dividing the measured radioactivity of a spot (after subtraction of background radioactivity) by the (sum of) chain length(s) of the corresponding oligonucleotide(s) present in that spot.

‡ The molarity is calculated as the ratio between the effective c.p.m. per nucleotide for a given spot and the standard c.p.m. per nucleotide; the latter is the average of the c.p.m. per nucleotide values for all the listed oligonucleotides ($n > 4$) present in the *Hind* II+III fragments E, K, F, J and G.

§ Each oligonucleotide present in the RNA could be unambiguously assigned to a *Hind* II+III fragment from the late region of the genome on the basis of the known DNA sequences (see Fig. 1); a dash connecting two fragments indicates that the oligonucleotide concerned overlaps the two *Hind* II+III fragments.

¶ Double-digestion analysis with pancreatic RNase revealed that several individual components or sequence isomers were present.

** This spot appears weaker in Fig. 3a because the radioactivity is spread over a larger area.

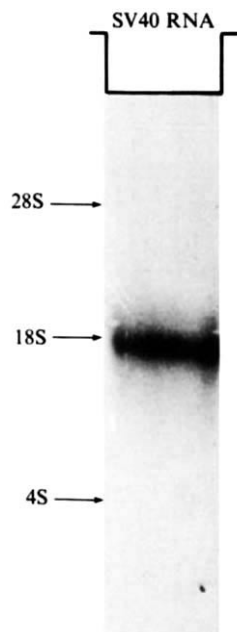


Fig. 2 Purification of ^{32}P -labelled late SV40 mRNA on 1.4% agarose gel. ^{32}P -labelled, polyA-containing RNA was extracted from SV40-infected CV1 cells and hybridised onto Sepharose-bound SV40 DNA. The virus-specific material was eluted, briefly heated and directly loaded onto a 1.4% agarose gel for electrophoresis in 20 mM Tris-citrate, 1 mM EDTA, pH 7.5, at 50 V for 3 h at 0°C. The ^{32}P -labelled RNA was localised by autoradiography. The arrows indicate the position of the rRNA markers run in parallel lanes (note that rRNAs are relatively less retarded in agarose gel electrophoresis such that 18S rRNA moves to about the same position as 16S mRNA)³.

although they were also present in about molar amounts in the fingerprints of each SV40 16S mRNA preparation (Table 1). They could be correlated unambiguously, however, with a DNA segment more counterclockwise in the late region of the genome and spanning the *Hind* II+III

fragments C (right end), L and M (map coordinates 0.72 to 0.76; see Figs 1 and 4). We also characterised the SV40 16S mRNA by digestion with pancreatic RNase and fingerprinting¹⁸. All the potential pyrimidine-terminal products were again identified, those from both the coding part of the gene and the 3'-untranslated segment as well as those from the approximately 40 nucleotide segment before the initiation codon and those from the 0.72–0.76 leader sequence region. These results further strengthened our conclusions on the sequence representation in the major, 16S mRNA.

No large oligonucleotides from *Hind* II+III fragment D were detected in 16S RNA, while all the products (T1 and pancreatic oligonucleotides) from *Hind* II+III fragment M were clearly present; hence we believe that the putative 3'-terminal 'splicing' site of the leader segment is located close to the *Hind* II+III restriction site between fragments M and D. Correct positioning of the 5' end, and hence of the whole mRNA, was not yet possible, mainly because the sequence in that area lacks discriminative T1 and/or pancreatic oligonucleotides (Fig. 4). The T1 product UUAUUUCAG, preceding the *Hap* (or *Hpa* II) site by 14 nucleotides (ref. 21 and H. Van Heuverswyn, personal communication), was not present in the 16S RNA. However, as the aforementioned AUU sequence occurs in it, the oligonucleotide UUAUUUCAG may possibly correspond to the region where cap formation takes place¹¹. Alternatively, the 5'-leader sequence may be generated by two splicing events and the 5'-terminal cap oligonucleotide would then be derived from a region still more counterclockwise on the genome. It is remarkable, however, that a number of viable deletion mutants have been reported which lack up to 50% and perhaps more of the 5'-leader fragment here characterised^{22,23} (work is in progress in our laboratory to delineate more precisely the deletion of such mutants by actual DNA sequencing).

In conclusion, we have shown that the major SV40 mRNA is coded for by at least two separate regions on the genome. The 5'-leader sequence is about 180 nucleotides in length and is transcribed from the region 0.72 to 0.76 on the standard map. The body of the mRNA starts at most 40 nucleotides before the initiation codon for the VP1 protein and contains the whole structural VP1 gene followed by the untranslated 3'-terminal end; it thus extends clockwise



Fig. 3 Two-dimensional fractionation of a T1 digest of 16S SV40 mRNA. The RNA was prepared and isolated as briefly described in the text and digested with 3 units of ribonuclease T1 at 37°C for 15 min. The hydrolysate was applied directly onto a pre-wetted cellulose acetate strip for high voltage electrophoresis at pH 3.5 in the presence of 5 M urea. The material was then transferred by reversed blotting onto a polyethyleneimine plate and developed in the second dimension with homomixture β at 60°C (ref. 18). As the first dimension separates according to base composition and the second dimension according to chain length, the composition of nearly every spot can be deduced from its position. *a*, Autoradiograph of an original fingerprint. *b*, Schematic diagram identifying the oligonucleotides present. The latter are indicated by a standard nomenclature which is based on the nucleotide composition of the spots, as explained by De Wachter *et al.*³⁸; the numerals refer to the U, C and A content respectively. *c*, Schematic diagram (corresponding to *b*) distinguishing between the digestion products derived from the body of the 16S mRNA (white spots) and the oligonucleotides which are unique to, and hence specific for, the 5'-terminal leader segment of the mRNA (black spots). Additional unique oligonucleotides, present only in the fingerprint of late 19S mRNA, are indicated by dashed circles. They are hardly visible on the original autoradiograph of the 16S fingerprint (*a*) and represent less than 10% of the radioactivity expected for a molar ratio.

from coordinates 0.94 to 0.17 (Fig. 1). There is no evidence that the two parts of the RNA molecule are joined by anything other than a normal phosphodiester linkage. It is at present not known whether the splicing occurs during transcription (a 'gliding' RNA polymerase) or is the result of a subsequent excision-ligation event. The former hypothesis is unlikely in view of the results with adenovirus-infected cells, where a full transcript appears to be the

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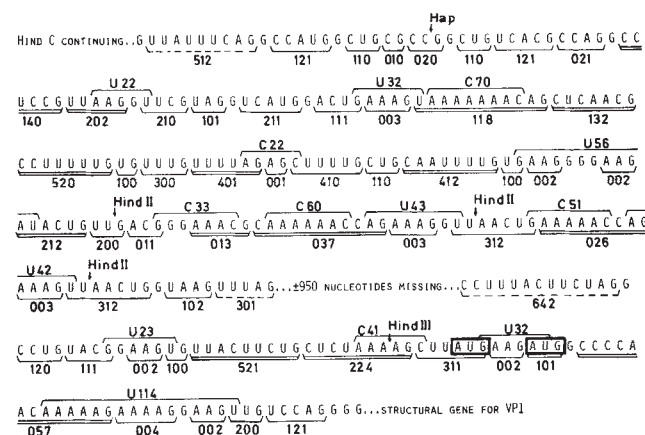


Fig. 4 Partial nucleotide sequence of the untranslated 5'-terminal leader sequence of SV40 16S mRNA. The RNA sequence has been deduced from the corresponding DNA sequences of the different *Hind* II-III restriction fragments of SV40 DNA (reading in clockwise direction): the last 149 nucleotides of *Hind* II+III fragment C (ref. 34 and H. Van Heuverswyn, personal communication), *Hind* II+III fragments L and M³⁵, the first 15 nucleotides of *Hind* II+III fragment D³⁶, the last 44 nucleotides of *Hind* II+III fragment E²⁰, and the first 48 nucleotides of *Hind* II+III fragment K³⁷. The numbers of the TI products refer to their base composition (see legend to Fig. 3, Table 1 and ref. 33). Doubly underlined oligonucleotides are unique and therefore discriminative products certainly present in the fingerprint (Table 1); singly underlined oligonucleotides are also observed but their presence is not discriminative. Dotted lines denote characteristic oligonucleotides which are predicted from the DNA sequencing data but which were not detected in the mRNA fingerprint. An analogous system of numbering is used for the pancreatic RNase oligonucleotides³⁸; characteristic products certainly present in the fingerprint are indicated by lines above the sequence. Initiation of translation of the VP1 gene can start at either of the two AUG triplets shown in boxes⁸, but recent *in vitro* translation experiments favour the 5'-proximal AUG triplet (A. Mellor, R. Hewick, M. Waterfield and A. Smith, personal communication).

precursor to the late mRNAs²⁴⁻²⁶, and considering that also in SV40 the 16S mRNA may be derived from a larger precursor²⁷. Splicing by the latter mechanism may involve a base-paired stem, where the cutting-ligating enzyme(s) would act; however, we could not yet identify extensive base-pairing in the relevant region. The concept of splicing in the formation of eukaryotic mRNA may perhaps partly explain the complex processing events undergone by heterogeneous nuclear RNA and may form the basis of a new type of control for the expression of the genetic information.

After this manuscript was submitted results were published which were obtained by nucleic acid sequencing²⁸, nucleic acid hybridisation^{29,30} and electron microscopy³¹ and which lead to the same general conclusions as reported here. More detailed information, however, is revealed by our characterisation of the mRNA nucleotide sequence, as summarised in Fig. 4. Also, the complete nucleotide sequence of SV40 DNA is now known^{21,32}.

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matters arising

Assessment of risk of sudden death in infants

THE study by Carpenter and Emery¹ into the incidence of sudden death in children raises several methodological questions. They have clearly demonstrated that their classification into high- and low-risk groups works, but their evaluation of surveillance is less convincing.

Carpenter and Emery set out to assign children randomly to observed and control groups, yet children born at certain periods (holidays) were all assigned to control. While there may be no reason to suppose that these children are different from others, there is equally no reason to suppose that they are not. They should have been excluded from the analysis altogether if they could not be assigned to the observed group.

In addition, Carpenter and Emery failed to demonstrate a significant difference between observed and control groups. This is not surprising, as such failure was built in to the design of the study. If the proportion of deaths in that 'at risk' population were that found in the control group, 9.8/1,000, and the treatment reduced this to 1/1,000, then the chance of finding a significant difference at the 5% level would be 70% (ref. 2). If the mortality were reduced to 3/1,000, as in the sample, the chance of finding a significant difference would be less than 50%. Even if the treatment were effective, it would be very unlikely it could be demonstrated.

Having failed, for the above if for no other reason, to demonstrate a significant difference between observed and treated groups, Carpenter and Emery combine those who were assigned to treatment but who refused it with the control group. There is no reason to suppose that those who refused are comparable to those who accepted, indeed, it would seem unlikely that they are. The refusers should be combined with the observed if we are to be true to the intention of the randomised trial. This gives a death rate of 6.0/1,000 among the observed group to compare to 9.8/1,000 in control, a difference which could easily have arisen by chance.

There seems to be no justification for any service innovation in this study. However, the findings so far

are certainly worth pursuing, and re-analysis as suggested, combined with a further study period involving more children, would answer the question one way or the other.

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CARPENTER AND EMERY REPLY—Bland¹ raises several points requiring comment. His conclusion that "there seems to be no justification for any service innovation in this study" arises from applying the theory of hypothesis testing to a decision problem. Using his approach, one starts with the most obvious null hypothesis, regardless of how implausible it may be, and acts on the alternative hypothesis only when the null hypothesis is rejected, irrespective of the consequences of a wrong decision. It is a simplistic approach to statistics that is widely taught but can be out of touch with the problems of clinical decision making which relate to matters of life and death.

Our study^{2,3} establishes, as Bland agrees, that a high-risk group of infants can be identified at birth. In view of the 63% greater mortality in the untreated group compared with the treated group can we reasonably withhold increased surveillance from all high-risk infants? We have shown that the balance of probabilities is in favour of surveillance although the difference is not statistically significant¹. Our health visitors discovered critically ill infants some of whom would almost certainly not have survived but for prompt admission to hospital³ and, after surveillance of all high-risk infants, mortality was further reduced to almost exactly the predicted level³. In retrospect, the two deaths in the study group could probably have been prevented had our observations been followed by more determined intervention. The weight chart of the first 13-week-old infant showed a marked fall in the

rate of growth in the preceding 4 weeks³. The other child had been in hospital for failure to thrive and had been discharged too soon.

A substantial proportion of infants have symptoms in the 3 weeks before unexpected death and increased surveillance along the lines of our study could provide active medical care (in preparation). In Finland⁶ and Sweden⁷ the post-perinatal mortality rates were 3.7 and 2.9% in 1974 compared with 7 per thousand in England and Wales. In both countries more than 98% of infants are served by child health centres, which average more than eight contacts in Sweden and more than 10 contacts in Finland per child in the first year of life; both rates are apparently much higher than for England and Wales. In France infant mortality has declined since child benefits were made dependent on medical examinations. These observations point to the importance of surveillance⁸. In the light of such evidence we think it would have been unethical to prolong the controlled study in Sheffield⁴.

Our records show that randomisation was suspended only for 8 d in the Spring of 1973, affecting 16 high-risk infants none of whom died. Between 7 July and 14 September 1974, to avoid overloading the health visitors, only two in five high-risk infants were allocated at random to the study group.

Having advised us to discard some of our data, Bland criticises us for starting a trial with virtually no hope of success. This attack is based on our results, as though we had known what these would be, and ignores our prior information some of which we have reported³. Our prior information was that there were between 40 and 50 unexpected infant deaths in Sheffield per year (more than 8,000 births per year; post-perinatal mortality rate 8.5%, giving an unexpected death rate of about 6‰). The scoring system was planned to include half these deaths in the high-risk group. Our primary object^{3,9}, was to obtain prospective data on infants before they died. Surveillance is seldom without effect, so that a high-risk control group was essential both to evaluate the scoring system and to obtain a measure of the effects of the surveillance. We did not anticipate the considerable decline in rates of birth and mortality, the combined effects of

which left us with only nine deaths in the high-risk control group, instead of the 20 expected.

We are taken to task for reporting that on the most favourable view possible of our results, that is when the refusals are grouped with controls and using a one-sided significance test, the difference between the number of deaths in the two groups does not reach the conventional level of statistical significance. It follows that if this difference is not statistically significant, no other can be. We are left with the balance of probabilities in favour of surveillance. Calculations like Bland's show that the mortality rate in Sheffield is now so low that, on present figures, an intervention trial with a 90% chance of success would take 20 yr to complete.

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Circular structures of large scale and great age on the Earth's surface

SAUL¹ recognises large circular structures on the Earth's surface. We broadly agree with him as to their nature but must point out that such circular patterns have been described before. The existence of similar structures was demonstrated at the Geological Society of London in 1964, after the presentation of a paper by Kellaway entitled: Structural rings and fissure systems in relation to river development². Detection of the ring structures (called non-volcanic cycloliths) was achieved initially from detailed topographical maps, later supplemented by aerial photographs and geological information, while the presence of spiral joint patterns within some non-volcanic cycloliths was noted by Durrance³ and the coincidence between joint and drainage patterns later confirmed⁴. We are currently compiling a map of the British Isles showing the position of

the non-volcanic cycloliths so far recognised.

Some British non-volcanic cycloliths are sufficiently well defined topographically to be recognisable even in the geological map of Great Britain⁵. An example, with a diameter of about 50 km, is that at Chipping Norton (centred at National Grid Reference SP 2525). This area consists of dissected plateaux of Inferior and Great Oolite limestones, where the underlying Lias is exposed in the valley floors. Erosion has clearly followed a general circular pattern, picking out a fracture system of the same configuration.

We agree with Saul that some of the non-volcanic cycloliths could owe their origin to meteorite impactation at a very early date in the Earth's history, but a possible cyclolith of relatively recent age is the Richat structure in Mauritania⁶. Also the possibility that some owe their formation to an internal mechanism cannot be excluded.

Whatever their origin it is clear that cycloliths may be of fundamental importance to the assessment of many geological processes affecting the Earth in the last 4000 Myr.

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SAUL REPLIES—The circular features which I described¹ are common on the North American continent and probably have a worldwide distribution pattern. It is expected and desired that these structures, now that they have been characterised, should be recognisable and of use in reinterpreting earlier geological observations and in carrying out future work.

The structures of Kellaway and Durrance² are “circular or elliptical”³ and were recognised by jointing and drainage patterns only in areas of relatively flat-lying sedimentary cover. Although the descriptions are far from identical, it is possible that the structures of Kellaway and Durrance belong to the same class of feature as the circles I described, and that the differences are due to aspects of the local geology and topography. I have previously speculated that the (easternmost) long curved water course shown

in the rather flat northeastern section of Fig. 1 of my original paper¹ was part of “a circular rim with negative topography”. This water course should perhaps be compared in detail with the Chipping Norton structure, whose existence and general circularity are not in doubt.

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Timing of continental growth and emergence

WINDLEY¹ cogently summarises evidence based on crustal thickness and on the existence of major sedimentary basins of early Proterozoic age to counter the arguments of Hargraves² purporting that continents did not emerge from the sea until ~1,400–1,000 Myr ago. Indeed, the oldest known terrestrial sediments, namely the ~3,800 Myr-old Isua supracrustal sequence of West Greenland, contain major conglomerate horizons and boulder beds^{3–5}, indicating the presence of land.

In my view, however, Windley¹ overstates his case by suggesting that “there is increasing evidence that the growth of the present-day continents, both in terms of surface area and thickness, was probably almost complete by 2,500 Myr ago”. He quotes isotopic evidence ranging from ~50% to 100% for proportion of present-day continental crust already in existence 2,500 Myr ago, but arbitrarily prefers the second value. Without wishing to denigrate the importance and worldwide extent of the ~2,800–2,500-Myr continent-forming event, which I have frequently emphasised (for example, ref. 6), I believe it to be premature to propose that continental growth was all but complete by 2,500 Myr ago. The two extreme estimates are, of course, contradictory and would lead to quite different hypotheses for continental evolution.

Windley's proposal implies that since 2,500 Myr ago the rate of destruction of continental crust has equalled its rate of formation and that subduction-related processes have effectively and completely mixed continental crust with the mantle, so that the resulting homogeneous mixture presumably forms the source for new continental crust. This view is favoured by some geologists^{7,8}, whereas others prefer the view that continental crust has been produced by irreversible differentiation of the mantle

through geological time^{8,9,10} by mechanisms also interpretable in terms of global tectonics.

Apart from the mechanical difficulties in quantitatively subducting continental detritus¹¹ and then mixing it completely with the mantle, there is much geochemical and isotopic evidence that the source regions of oceanic basalts have been heterogeneous for ~1,500 Myr or more^{12,13}. Considerations of diffusion parameters suggest that large-scale mantle mixing is implausible¹⁴. Furthermore, in the absence of selective crustal isotopic contamination, isotopic data from many Phanerozoic and modern island-arc and continental margin calc-alkaline igneous rocks clearly indicate an origin from source regions within upper mantle and/or subducted basic lithosphere with little or no contribution of continental detritus with average crustal Rb/Sr values^{15,16}. Indeed, this seems to be consistent with a recent paper by Windley and Smith¹⁷, in which close analogy is drawn between igneous rocks formed at modern destructive plate margins and high-grade gneiss complexes of Archaean age.

I interpret much of the evidence to suggest that the bulk of juvenile continental crust has been produced throughout geological time (possibly episodically) from mantle, or basic lithosphere, that have not previously mixed to any significant extent with continental crust^{8,18}. It is likely that the inconsistent evidence quoted by Windley¹ will soon be superseded by age and isotope data capable of yielding a truly quantitative measure of continental growth in any given area, as foreseen in the pioneering work of Hurley *et al.*^{19,20}.

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Failure of acids to eliminate aziridinyl residues in chemosterilised mosquitoes

THIOTEPA and several of its analogues chemosterilise male mosquito pupae, but the use of these compounds in the field has been restricted because of the presence of mutagenic residues inside the pupae. We were therefore interested in Sharma's¹ proposal for eliminating mutagenic residues of chemosterilant inside mosquito pupae. Providing no chemical documentation, he proposed that chemosterilised pupae should be soaked in 0.0025 N H₂SO₄ for 90 min, and based his proposal on the unstable nature of thiotepa (and its analogues) in media of low pH. We investigated the proposed method by using a gas chromatograph equipped with a flame photometric detector to confirm the levels of residues of an analogue of thiotepa in chemosterilised pupae after treatment in H₂SO₄. We found that the method had no detectable effect on residues of the chemosterilant inside the pupae.

We used a 1% aqueous solution of *P,P*-bis(1-aziridinyl)-*N*-methylphosphorothioic amide (referred to as A13-61585) to sterilise pupae of the mosquito *Anopheles albimanus*. Exposure of pupae for 1 h in a 1% solution consistently induced more than 99% sterility in males of *A. albimanus*². We used the methods of Seawright *et al.*³ to rear mosquitoes, extract the residues and conduct gas chromatographic analysis. The column (4mm inner diameter) was packed with 3% OV-225 on Gas Chrom Q 100–120 mesh. A flame photometric detector was operated in the phosphorus mode using a 525-nm filter and a solvent bypass valve.

In preliminary experiments, we noted that formulation of a 1% solution of A13-61585 in 0.01 N H₂SO₄ caused complete degradation of the chemosterilant within 10 min. This observation was consistent with Sharma's bioassay results¹. However, there was no quantitative difference in the residues of A13-61585 in chemosterilised pupae soaked either in 0.01 N H₂SO₄ for 1 h or in distilled water.

In further tests the chemosterilised pupae were soaked in 0.1 N H₂SO₄ or 0.1 N HCl for 1–4 h. The internal residues of A13-61585 were statistically equal in the acid-treated and control pupae. As there was no apparent degradation of chemosterilant within the pupal tissue, we added up to 1,000 p.p.m. of dimethylsulphoxide (DMSO) to a few acid solutions in an attempt to enhance penetration of the insect cuticle and to decrease surface

tension. However, the addition of DMSO had no effect on internal residues of A13-61585.

Our final experiment with 0.01 N and 0.1 N H₂SO₄ is summarised in Table 1, and the data are representative of all our observations. Soaking the chemosterilised pupae in acidic media clearly had no effect on levels of internal residues of A13-61585. The acid probably does not penetrate the cuticle of the pupae because of the highly polar nature of strong acids. We feel that similar observations would be obtained with closely related analogues of A13-61585, such as thiotepa.

Table 1 Amounts of A13-61585 found in pupae of *Anopheles albimanus*

Treatment time (h)		ng A13-61585 per pupa	
0.1 N H ₂ SO ₄	0.1 N H ₂ SO ₄	H ₂ O	
2	1		40.3 ± 4.9
			52.2 ± 2.5
	3	1	49.3 ± 7.2
		3	38.0 ± 4.8
			34.0 ± 6.8

Pupae were treated in a 1% solution of the aziridinyl sterilant for 1 h and then held in H₂SO₄ (0.01–0.1 N) or H₂O for 1–3 h. Each mean is the average of three samples each with 200 pupae.

Although our data contradict Sharma's conclusion, the idea of using a chemical to degrade chemosterilant residues deserves more inquiry. There are two necessary requirements for such a compound; first, it must penetrate the cuticle of a mosquito pupa, and second, it must react with the chemosterilant to produce innocuous products. Penetration of the cuticle is related to the polarity of the compound, and relatively non-polar substances penetrate to a greater extent than do polar substances. For degradation of the chemosterilant, the compound should have groups that react with the ethylencimine moieties. We have initiated a screening programme to find a compound that will perform these two functions without severe impairment of the vigour of chemosterilised males.

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reviews

Palaeontological galaxy

George Gaylord Simpson

Patterns of Evolution, as Illustrated by the Fossil Record. Edited by A. Hallam. Pp. xiii + 591. (Elsevier Scientific: New York, Amsterdam and Oxford, 1977.) Dfl.170; \$69.50.

THE science of palaeontology is commonly divided into the specialities of micropalaeontology, invertebrate palaeontology, vertebrate palaeontology and palaeobotany. Palynology, currently an active subfield, is micropalaeontological in method and palaeobotanical in subject matter. Until relatively recently, micropalaeontology and invertebrate palaeontology have been pursued primarily as adjuncts of geology for stratigraphic purposes, broadly speaking. Vertebrate palaeontologists, from early in the nineteenth century down to the present, have usually been more biological, including concern with such subjects as anatomy and biogeography, and since Darwin with evolutionary theory. Palaeobotany, with fewer practitioners, has had broad but less clearly focused concerns. In recent years, the invertebrate palaeontologists especially have become more biological in outlook and have joined the vertebrate palaeontologists in what might be called the principles and practice of historical biology. The present volume is an outcome and illustration of that development and a major contribution to it.

In addition to a brief but cogent preface by Hallam, the volume has seventeen chapters by seventeen authors. All the authors are established and productive scientists and they form an international palaeontological galaxy: ten now working in the United States, four in Great Britain, and one each in Canada, Denmark, and the German Federal Republic. The first three chapters, by Gould, Valentine and Raup, and the last by Schopf, are general in nature. Seven chapters treat taxonomically defined invertebrates: Williams and Hurst on brachiopods, Paul on echinoderms, Schopf on bryozoans, Stanley on bivalves, Kennedy on ammonites, Eldredge on trilobites and Richards on graptolites. The roster of invertebrates is thus incomplete but does include the most significant groups in the fossil record. In general those are treated not only from a systematic point of view but

also for their possible contribution to evolutionary theory. All the major groups of vertebrates are discussed: fishes, in the broadest sense, by Thomson; amphibians by Carroll; tetrapods, as a whole but primarily reptiles, by Bakker; and mammals by Gingerich. Trace fossils, representing activities of invertebrates rather than the animals themselves, are considered by Seilacher. A single chapter on palaeobotany by Doyle is devoted mainly to early angiosperms and is largely palynological.

The historical and theme-setting chapter by Gould is the sort of virtuoso performance that one confidently expects from its author, but in this context it is rather inapposite. Gould's weakness for paradox has led him to a series of three theses and antitheses that are either belied or more often simply ignored by the other authors. For example, Gould belabours Valentine at some length for "radical environmentalism", but the immediately following chapter by Valentine himself refutes Gould's accusation (without mentioning it).

Raup's chapter on stochastic models is methodological in a quite general way. Besides illustrating various approaches to such modelling, Raup demonstrates that stochastic models do not have negation of causation as a premise but can help to determine when a causal explanation cannot be safely adopted, whether the causes are simply unknown or whether they are too complex for identification from the data in hand.

Williams and Hurst discuss brachiopod diversity and evolutionary rates in what may now be considered an orthodox way, but they devote most attention to details of morphological change, phylogeny and taxonomy. Here, already, appears a source of confusion that is common among both palaeontologists and neontologists and that recurs in many chapters of this book. No sufficiently clear distinction is made between phylogeny and classification. The reconstruction of phylogeny from data that are never complete is a constant preoccupation that generally has only a debatable outcome, but what is under consideration was a factual objective sequence in nature. Classification is not factual in that sense. It is always an artifact of the

human mind and it remains subjective and retains its artificial aspect even though most students relate their classifications in some way and to some degree to their concept of phylogeny.

Paul on primitive echinoderms ascribes the usual abrupt appearances of taxa in the fossil record to the incompleteness of that record, which is in the original sense a Darwinian point of view. Schopf again illustrates concepts of the current synthetic theory by examples from bryozoans, believing that both allopatric (cladistic) and phyletic evolution is involved and emphasising the effects of geographical distance.

After introducing some refinements into the estimation of rate of evolution and confirming the long previous conclusion that mammals have, on the average, evolved much more rapidly than bivalves, Stanley discusses what he calls "species selection" and contrasts this with Darwinian natural selection. Darwin did recognise, although perhaps not so clearly, that natural selection may be interspecific as well as intraspecific. "Species selection" is simply natural selection at a particular taxonomic level, but Stanley has emphasised well that major changes are best studied at this (or higher) levels.

Space will not permit even such brief and partial summaries of all the other chapters. It can be said that most of them fit in well with the synthetic theory of evolution and provide many special examples of evolutionary phenomena and their interpretation. Not only Paul, as previously mentioned, but also others, such as Carroll, emphasise that attention must continue to be given to the incompleteness of the fossil record and that this is not merely a 'gradualist' bias.

There are some partial exceptions to the generalisation that the authors propose only minor variations in the corpus of synthetic evolutionary theory. Bakker, as an instance of partial exception, refers to "punctuated equilibria" in tetrapod, especially dinosaurian, evolution, but this can be read as meaning that new groups appear suddenly in basins of deposition because they evolved (by whatever mode) in uplands of erosion. Bakker, by the way, might be characterised as at least a semi-environmentalist as regards his main

topic, mass extinction. In his final summarising chapter, Schopf proposes to follow Gould's triple theses—anti-theses, but even the summary strongly belies Gould's insistence that evolution has made no difference in these old "metaphors" (his term for them).

The one apparently almost total exception to the presence of a central body of varied but widely accepted principles is the chapter by Eldredge. He insists that "there are two fundamentally different ways of looking at the evolutionary process" and that before him palaeontologists have been following the wrong one. That wrong way is to think of evolution as morphological change. The right way is to think of it as speciation (the origin of new clades at the specific level). He says that neodarwinism (his historically ambiguous term for the synthetic theory) "is a nearly total admixture of

the two approaches", but he is definitely opposed to that school of thought. It is therefore surprising to find that when he gets down to discussion of his own speciality, the evolution and classification of trilobites, his treatment is likewise a nearly total mixture of the two approaches. Except for a few esoteric terms, what he has to say is quite "neo-darwinian" (syntheticist).

Eldredge's most extraordinary statement, however, is that until now palaeontology has made no substantive contribution to evolutionary theory. The whole book, including his chapter, adduces strong evidence to the contrary. □

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Islamic mathematics

The Muslim Contribution to Mathematics. By Ali Abdullah Al-Daffa'. Pp. 121. (Croom Helm: London, 1977.) £7.95.

THE same increase in world oil prices which has begotten militaristic extremes of nationalism in certain Arabic countries has in others led to a gentler resurgence of pride in their common language and Muslim culture, and of a fresh consciousness of their once-great past a thousand and more years ago when Islam held sway over half the world. There have, ever since our own European intellectual 'rebirth' in the sixteenth century with its heavy emphasis on the rediscovery of Greek thought and civilisation, always been scholars around who have been ready to give just appraisal of the Arabic achievement in science in its widest sense, but it has never seemed important for our historical textbooks to dwell more than fleetingly on this millennium of Oriental dominion between the passages of arms in ancient Greece and Rome and the wars and political passions of our modern Latin West. It is understandable that the Arabs, even as they pour some of their new-found riches into bringing themselves into line with so much in areas in which they had previously lagged behind, should also put money into making propaganda for their own past, stressing wherever possible its originalities and depths of vision. And indeed who has escaped a glimpse at least of the bandwagon of *The World of Islam* as it has recently creaked along in this country in one form or another? Unfortunately, the purblind amateurishness of those who ride trumpeting

upon it so often gainsays the attractive freshness of their enthusiasm for their cause. And so it is with this little book (quite outrageously priced for the half-dozen short essays which it comprises) from the pen of—so the dustjacket blurb informs—the Chairman of the Department of Mathematics at the University of Petroleum and Minerals, Dhahran, Saudi Arabia.

Al-Daffa' begins briskly enough by sketching the beginnings of Islam from the Prophet Mohammed through the Caliphates to the 'ruthless' Mongol invaders who broke up the central Muslim Empire in our mid-thirteenth century (no mention that those same pitiless heathens set up in their wake what was Islam's finest and most elaborate observatory-cum-mathematics centre at Samarkand . . .). Then come a dozen (or less) pages each on Arabic arithmetic, algebra, trigonometry and geometry, each supported by several pages of bibliographical notes (their contents are listed again alphabetically by author at the end of the book) where reference is made to such scholarly works on the Arabic endeavour as Hogben's *Mathematics for the Million*, Bernal's *Science in History* and numerous obscure American high-school mathematics textbooks. The kindest comment I have to pass on Al-Daffa's own scholarship is that it is shallow, basically uninformed and mostly out of date when it has a firm basis in fact. In arithmetic (which "the author believes [*sic*] is a vital part of daily living because it meets practical needs") he correctly singles out the importance of the Arabs' fashioning and refining of the Hindu system of numerals into effectively its modern form, with zero (*sifr*) as a place value, and its novel extension to embrace decimal fractions (but nowhere mentions the name of

Al-Kashi, who at Samarkand in the fifteenth century did most to advance numerical methods making use of the latter's power). In algebra he makes much of Al-Khwarizmi on the solution of quadratic equations, insisting much on the "*Regular [sic] Falsi*", the method of false position, which yields their numerical roots (but fails anywhere to mention the 'Mongol' Al-Tusi of Maragha who went on to duplicate the general resolution of the numerical equation which we Europeans know as Viète's). Of the Islamic achievements in number theory Al-Daffa' is especially proud, pointing to 'Thabit ibn Qurra's parametrisation of amicable numbers in the ninth century (though he cannot properly state it on his p44) and to the unidentified "Muslims" who first "discovered" Fermat's theorem that "for integers the sum of two cubes can never be a cube" (but he seems not to know that Al-Husayn, who reports to us this tenth-century guess of Al-Hughandi's, states firmly that he could not prove it).

And so I could go on. It fits in with the general quality of this new contribution to our European understanding of the Islamic achievement in exact science that the dustjacket reproduces (from an un-named manuscript) a figure illustrating "Ibn Al-Shatir's"—it is in fact Al-Tusi's dressed up—"theory [*sc.* 'Copernican' model] of planetary motion": one whose copyist has so little understood what it is he transcribes that he has drawn the primary epicycle fixed on the rotating deferent vector, producing a 'lunar orbit' with perigees at syzygies and apogees at quadratures.

It is my plain duty to tell anyone who wants an introduction to the Muslim achievement to go elsewhere, and leave this deplorable book gathering dust on the booksellers' shelves from which it ought never to be sold. For the same price or thereabouts, let me add, one may now buy the balanced and scholarly survey by A. P. Youschkevitch of *Les Mathématiques Arabes (VIII^e-XV^e siècles)* (Paris: Vrin, 1976; a corrected and much amplified version of the central chapter in his standard Russian monograph on medieval mathematics). You may be sure that Al-Daffa' does not know even Youschkevitch's name, or that of his colleague Boris Rosenfeld or of most other current non-Arabic workers in the field of classical Islamic mathematics. No manner of enthusiasm can make up for deep-rooted ignorance.

D. T. Whiteside

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Mammalian embryogenesis

Concepts in Mammalian Embryogenesis. Edited by Michael I. Sherman. Pp. 404. (MIT Press: Cambridge, Massachusetts and London, 1977.) £17.50; \$30.

"It is a tribute to the research enterprise currently directed towards the molecular biology of the early mammalian embryo that new chapters are being added to the unfolding story almost every year." So Van Blerkom and Manes begin their chapter. Every chapter could begin with a similar sentence. They go on: "We are aware of the numerous reviews . . .". So am I, and I was feeling fairly bored when I began reading. The boredom did not last long. This collection of papers is the most readable account of most of the very various lines of research currently forming the mammalian development bandwagon.

Sherman points out, somewhat apologetically, that the book is mostly about mice. There is no need to apologise; much comparative biology is included. The book covers classical embryology, genetics, molecular biology, cell surfaces (Jenkinson and Billington), tumour viruses (Jaenisch and Berns) and terato-

carcinomas (Graham). The chapters on embryology (Rossant and Papaioannou), and genetics of the early embryo (Chapman, West and Adler) add little if anything to the other recent reviews, principally because they involve the most time-consuming experimental analysis, but Rossant and Papaioannou include an uncommon but valuable historical perspective on the embryology.

There are positively heroic efforts to analyse two problems which make the Gordian knot look like a job for the Boy Scouts. Sherman and Wudl tackle the T-locus mutations and gather together, in 99 pages, a quite bewildering compendium of data which, although a bit repetitious, is an invaluable service in itself. They have brought conflicting data on this complex chromosomal site face to face in one paper—much of it, I think, for the first time. To follow all their arguments seems to me a job for six months' solitary confinement but at least it is a stimulating challenge to current dogma.

The second major contribution is Graham's review of teratocarcinomas. Though shorter (but only just), this chapter discusses the several origins of teratocarcinomas and attempts to resolve some of the interesting differences between the tumours produced, and the

differences between them and embryos.

All chapters are very well referenced and the editors have had the foresight to insist on a common terminology throughout, providing a glossary at the start of the book. A good idea, but it is unfortunate that they have elected to perpetuate a few confusing inaccuracies—for example, visceral endoderm for proximal endoderm, embryonic or primitive ectoderm for epiblast (this latter confusion by failing to provide guidance of any sort).

There are two curious omissions; parthenogenesis, which receives three paragraphs *in toto*; and embryos *in vitro* which, although very much in evidence throughout, do not receive specific mention to any extent. In the index one author apparently accords them ten pages but inspection reveals that seven of these are taken up by illustrations of other things.

But these criticisms are trivial, as the monograph is justified by the T locus and teratocarcinoma reviews alone; the remainder is high quality icing on the cake.

Michael H. L. Snow

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Medicinal plants

Major Medicinal Plants: Botany, Culture and Uses. By Julia F. Morton. Pp. 431. (Charles C. Thomas: Springfield, Illinois, 1977.) \$49.50.

Books on medicinal plants vary from folksy and occasionally somewhat batty popular ones to textbooks of pharmacognosy, and it may be difficult to realise that they are about some of the same plants. Dr Morton's book is not folksy and certainly has no affinity to the lunatic fringe, but neither is it a textbook; indeed, she states this specifically. It is, however, a valuable adjunct to textbooks of pharmacognosy and should be seen in that light.

About a hundred plants are arranged systematically with sections giving descriptions, origins and distribution, medicinal and other uses, constituents, propagation, and so on. A great deal of work has gone into accumulating the information, and most of it is both pertinent and interesting. However, one is brought up short on occasion by statements such as the one that Marsh Mallow (*Althaea officinalis*) is grown in gardens in Great Britain. No, I am afraid it is not. Possibly the reason for this error is too great a reliance on common names; according to Mrs Grieve in *A Modern Herbal* country people used to call the Common Mallow, Marsh Mallow. Again, the distribution of *Frangula alnus* (syn. *Rhamnus*

frangula) is given as South-Eastern Europe and Russia. In fact, the Alder Buckthorn is native to Britain and on the Continent from Sweden to the Urals and Siberia, as well as Morocco and Algeria! But these are minor quibbles, amongst a mass of useful information.

It is interesting to note how few plants are used medicinally in orthodox American medicine nowadays. This point is emphasised by the long tables at the end of the book, one of which is on plants no longer official in the United States, but still mentioned in textbooks and still used in other countries. The other table is on plants used as pharmaceutical aids and adjuncts, varying from algae used in the production of agar to flavourings. There is also a copious bibliography.

The illustrations to this book let it down badly. Many are taken from Bentley and Trimen's *Medicinal Plants* (1880), and Blair's charming drawings have suffered a most unfortunate sea change in the course of reproduction. The modern drawings are coarse in texture, but this again may be due to the method of reproduction. The black-and-white photographs are practically all bad and often out of focus. The colour photographs too are not good, and there was surely no need to have the entire photograph of the Great Scarlet Poppy (*Papaver bracteatum*) bright red in colour?

Rosemary Angel

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Science and society

Perspectives in the Sociology of Science. Edited by Stuart S. Blume. Pp. 237. (Wiley: London, Sydney and New York, 1977.) £10.95; \$21.

THE common or garden reader of *Nature* is unlikely to give this volume a second glance. All those long words, theoretical analyses, and citations of this or that authority would quickly warn him that he had blundered into somebody else's academic specialty, as remote as non-linear optics or reptilian haematology. Like an aborigine faced with an X-ray tomographic section he might take a long time to recognise it as an image of his very own self.

The earnest student of the sociology of science will also be disappointed if he comes upon this volume as he browses along the shelf in search of general guidance. To a zeroth approximation, it is little more than a collection of primary papers on the 'Science and Society' theme such as might be found in a journal such as *Minerva*, without coherence one with another. Dr Blume, in his introductory survey of "Sociology of Sciences and Sociologies of Science", really says, somewhat elaborately, that there is no overarching theory, and pluralism is the order of the day. This is wise; but it doesn't explain why these particular papers should be written specially for publication in a single volume between expensive hard covers. There is a suggestion that the aim was to emphasise 'external' factors in scientific activity—national culture, economic and industrial needs, historical trends, and so on, but only a pedantic ignoramus could assert the contrary.

Considered separately, however, these papers offer food for thought—even for the journeyman scientist or lay citizen. R. D. Whitley, for example, in "The Sociology of Scientific Work and the History of Scientific Developments", strongly emphasises the connection between such mundane "administrative" issues as the departmental structure of universities and supposedly intellectual factors within the cognitive core of an academic discipline. His categories of "restricted" disciplines (for example, physics) and "un-restricted" disciplines (for example, biology) like Kuhn's "normal" and "revolutionary" phases of science, look too schematic, and would probably not stand up to detailed investigation, but he is right to emphasise that differences in the degree of paradigm ordering in various sciences are reflected in the relations between individual research workers, students and teachers, professors and their colleagues, in those sciences.

Peter Weingart, under the vague title "Science Policy and the Development of Science", deals specifically with the history of environmental research and action

in the Federal German Republic. This account of the interaction of technical, administrative and popular developments admirably illustrates the dynamism of science in an advanced industrial society, although I get the impression that environmental concern in Germany in the late 1960s was, indeed, driven 'externally' by louder voices echoing from the United States.

Strangely enough, sociologists of science have not shown much interest in science as a personal vocation. Elzbieta Neyman, writing on "Scientific Career, Scientific Generation, Scientific Labour Market", sketches out a research program for a "social psychology of science" that would remedy this deficiency. I am sceptical of the ultimate outcome of her very elaborate and detailed survey scheme, but she touches with great human insight and psychological sensitivity on many delicate questions, such as changes of role and status with age, which cannot be "answered" but which deserve further, deeper study.

The paper by Louis Orzack on the competition between pharmacists and other scientific professions for licensing as "quality controllers" of drugs in the EEC is only of interest as an item of historical evidence, and the account of "Nationalism and Nationalization of the Scientific Field in Quebec" is too brutally cut to fit marxian interpretations.

Fascination for social insects

Production Ecology of Ants and Termites. (International Biological Programme, Vol. 13.) Edited by M. V. Brian. Pp. 409. (Cambridge University Press: New York, Melbourne, Cambridge and London, 1977.) £19.50.

THIS volume in the International Biological Programme (IBP) Series results from the working group on social insects which was set up within the IBP section concerned with the productivity of terrestrial communities. As Professor Cragg points out in his preface, the theme had to be narrowed to ants and termites, and as Dr Brian the editor says somewhat plaintively "... the writers are intensely occupied with their investigations and could only be persuaded, with great difficulty, to write anything at all between spells in the field." In truth, the volume is more a collection of essays on ant and termite productivity and its measurement, than the results of coordinated international research.

The book is arranged in ten chapters, with fifteen contributors from eight countries, and this inevitably

And I wish that Radhika Ramasubban had integrated the general theory of her beautifully incisive essay "Towards a Relevant Sociology of Science for India" with her accurate perception of the depressing realities.

By this time, I had become rather bored with all those not quite compatible re-statements of the current state of the sociology of science. But being a conscientious reviewer, I persevered to the end—and found a real gem. Stephen Hill's summary of the true history and actualities of a major applied scientific research institution in a developing country should be read by every scientist and would-be science policy-maker concerned with the Third World. The cognitive dissonances, the sociopolitical mismatches, that turn the infinitely productive research method of the European industrial culture into an item of useless consumption, akin to a cargo cult, in countries such as Thailand, are bringing to naught one of the great aspirations of our times. As in all human activities, greed, pride and folly pay their parts: but the blame rests on us all, scientists and sociologists alike, for not trying to understand, and state clearly the nature of the peculiar enterprise in which we so naively engage.

John Ziman

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results in some variability in standard and presentation. The chapters, all in English, cover the collection of population data, with an introduction to production, followed by detailed reviews of food and feeding habits in termites and ants. A lengthy and painstaking summary of respiration and energy flow studies is followed by chapters on the nutrient dynamics of ants and termites, and their roles in ecosystems.

Everyone has their pet fascination when it comes to social insects which other authors always seem to underplay, and I would have liked more attention given to the dominant role of ants in the ecology of tropical trees, where an 'ant mosaic' is formed, each dominant species uniquely affecting the fauna of its tree by encouraging certain species (especially homoptera) and predating others. However, this book is a most valuable compilation of work to date (there are over 1,100 references), a guide to research techniques, and a source of challenging ideas for the future.

J. M. Cherrett

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Seed-eating birds

Granivorous Birds in Ecosystems. (International Biological Programme, Vol. 12.) Edited by J. Pinowski and S. C. Kendeigh. Pp. 431. (Cambridge University Press: New York, Melbourne, Cambridge and London, 1977.) £19.50.

THIS book describes the results of studies made under the auspices of the International Biological Programme, which was set up in 1964 as a counterpart of the International Geophysical Year, to encourage research within the theme of "the biological basis of productivity and human welfare". It arose because the rapidly expanding human population, and the increasing destruction of natural environments, called for more understanding of biological productivity and of how to manage natural resources without destroying them.

Some seed-eating birds are important to man because of their widespread occurrence as major crop pests. The chief subjects of this book are the House Sparrow *Passer domesticus* and the Tree Sparrow *P. montanus*, but it also covers the Red-billed Dioch *Quelea quelea* of Africa, and the Red-winged Blackbird *Agelaius phoeniceus*, Common Grackle *Quiscalus quiscula*, Brown-headed Cowbird *Molothrus ater*, Dickcissel *Spiza americana* and Horned Lark *Eremophila alpestris* of North America. The book presents a synthesis of ten years of co-ordinated research on these birds, and has been written by 12 scientists from North America, Europe and the Soviet Union.

Some of these birds are now among the best known in the world. Their abundance and status as pests have enabled large numbers to be collected for study. In consequence, certain chapters in the book are likely to serve as models for future studies. The introduction of the House Sparrow to North America during the last century, and the subsequent documented spread of the bird through a range of environments, has provided an unparalleled opportunity to study adaptation to local conditions, and has revealed some of the fastest rates of evolutionary change recorded in wild vertebrates (R. F. Johnston and W. J. Klitz). The chapter on avian energetics (S. C. Kendeigh, V. R. Dol'nik and V. M. Gavrilov) is the most up-to-date account in this field and provides a sound basis for further work. The chapter on pest management (M. I. Dyer and P. Ward) has application well beyond seed-eating birds. Other chapters deal with such topics as population dynamics, biomass and production, and adaptations of seed-eating birds and their role in the environment.

Perhaps the strongest feature of the book is the thorough integration of experience and ideas from widely separ-

ated parts of the world, reflecting the close cooperation between the authors at all stages of the project. One result of this is that regional studies can immediately be put into a wider context; for the first time the ecology of some of these birds is discussed over the range as a whole, rather than in particular localities (M. I. Dyer, J. Pinowski and B. Pinowska). Another result is that seemingly trivial findings of local studies are immediately revealed as widespread trends. Thus all the main avian pests of grain crops show tendencies to occur in huge aggregations; concentrate rapidly in areas of abundant food and disperse again when conditions change; breed rapidly; and resist the usual methods of control. Together with a seed-diet, these are the features that predispose certain species to become pests, when cereal monocultures are grown within their range.

Damage control schemes around the world also have certain tendencies in

common: they are usually based on exaggerated estimates of damage; they are started in response to political pressure from farmers, rather than from any objective assessment; control is usually based on killing the pest in large numbers, often costs more than the damage itself, and has adverse effects on other animals; and even when these facts become apparent, control is often continued, again in response to political pressure from crop growers. Often the damage to the total crop is negligible, but it is concentrated on the land of a few farmers or small communities.

In conclusion, this book provides a well-integrated synthesis of international studies on sparrows and other grain-eating birds. It is a pity that the book is so highly priced.

I. Newton

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Prostaglandin synthesis

Synthesis of Prostaglandins. By C. S. Szántay and L. Novák. Pp. 262. (Akadémiai Kiadó: Budapest, 1978.) \$16.

UNTIL very recently, scant attention had been paid to the problem of reviewing the vast field of prostaglandin synthesis. This timely book by Professors Szántay and Novák is the second independent work entitled *Synthesis of Prostaglandins* to appear in recent months (see *Nature*, 23 February, 271, 787; 1978) and seems, extraordinarily, to complement the previously reviewed volume in many areas.

The first chapter is devoted to a very detailed description of prostaglandin structure and nomenclature, followed by a generally excellent section on physical properties, particularly ^1H nuclear magnetic resonance and electron-impact mass spectrometry. However, a discussion of the controversial subject of prostaglandin conformation is followed by the odd statement, apparently based on the theoretical Hoyland-Kier harpin model, that, with regard to analogue synthesis, "no modification . . . counteracting the dispersive interaction between the side-chains is worth trying"; presumably, prostacyclin is thus expected to be biologically inactive. A brief but clear discussion of the major reactions and interconversions of prostaglandins concludes the section.

Chapters 2 and 3 outline the development of the major stereocontrolled synthetic approaches to the prostaglandin family, describing a number of examples in detail. These two chapters contain some stimulating and informative discussions of the classic problems of prostaglandin synthesis, of which the

excellent analysis of the development of stereoselective reduction methodology for the 15-carbonyl to 15-S-hydroxyl conversion is an example. On the debit side, the mechanistic interpretation of some of the synthetic reactions is too brief to be of much value.

Chapter 4 outlines the concepts behind the development of asymmetric prostaglandin syntheses of both chemical and biochemical type. The lucid explanations are only slightly marred by occasional ambiguities in the diagrams. Chapter 5 is a summary of approaches to some modified prostaglandins, including oxo-, thio- and aza-analogues; as this section is stated not to be comprehensive it may be of limited value. Finally, Chapters 6 and 7 contain summaries of biosynthesis, isolation from natural sources, and the analytical chemistry of prostaglandins.

The book is printed on rather poor quality paper and, although the typeface is clear, the diagrams are perhaps a little small for easy scanning. There is, of course, the occasional inevitable error in terminology ("formolysis" instead of "thermolysis") but generally the script is written in good and readable English. For the specialist synthetic chemist, the two main criticisms must be that the coverage is not comprehensive and that the referencing and indexing are very sparse; once again in this field the patent literature is completely neglected. The coverage is, however, quite up to date, although prostacyclin is only just included. Taken as a whole, the book will make a very readable and valuable addition to the literature of prostaglandins and should be of benefit to all those interested in the field.

C. J. Harris

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Record of oceanographic achievement

A Voyage of Discovery. (George Deacon 70th Anniversary Volume.) Supplement to Deep-Sea Research and Oceanographic Abstracts. Edited by Martin Angel. Pp. 696. (Pergamon: New York, 1977.) \$72; £40 (subscribers to Deep Sea Research, \$45; £25).

This volume forms a highly appropriate tribute to a remarkable man or, if Sir George Deacon would prefer it put in this way, then to a most remarkable record of achievement.

When, in 1972, we celebrated, most appropriately in Edinburgh, the centenary of the sailing of the *Challenger*, stock was naturally taken of British achievements in oceanography since that date. Then and for years afterwards Great Britain was the centre of deep-sea research, the high peak being represented by the completed publication of the *Challenger* Reports. But by that date an all-important influence had been lost.

As Margaret Deacon tells us in the initial contribution, the interest of the Admiralty in oceanographic research in 1880 and 1882 was under the direction of Staff-Commander Thomas Henry Tizard who had been navigating officer on the *Challenger*. After requests by Wyville Thomson, further observations were made in the Faeroe-Shetland Channel in the hope of determining the reasons for the striking differences in deep-sea temperatures observed there by himself and W. B. Carpenter during pre-*Challenger* expeditions in the *Lightning* and the *Porcupine*. An initial voyage in the shockingly unsuitable *Knight Errant* in 1880 was followed two years later by another on the *Triton*. On the basis of earlier published correspondence between Thomson and Tizard and of information contained in Tizard's journals in the possession of the National Maritime Museum, we learn how and with what difficulties in the worst of weathers (though this was in the months of July and May) the existence of the predicted Wyville Thomson ridge was fully established in 1882.

By that time the mantle of Wyville Thomson had fallen on the broad shoulders of John Murray but his hopes of continuing deep-sea investigations, which depended on continuance of the Admiralty cooperation maintained over the previous 14 years, were to be disappointed. To quote Margaret Deacon's final words, "This field, however, was to remain almost completely closed to British scientists until the



establishment of the *Discovery* investigations 10 years after Murray's death".

This brings us up to modern times, as it appears to one who then unsuccessfully sought appointment. What the Admiralty had previously neglected now became possible because of the surplus of money received for the use of the whaling station at South Georgia (this was before the advent of factory ships rendered shore facilities unnecessary). These investigations, under the initial direction of Stanley Kemp, were of immense significance both in their own right and in their effect on the development of oceanography in Great Britain. George Deacon participated in the second commission in R.S.S. *William Scoresby* in 1927–28 and in three of the first four commissions of R.R.S. *Discovery II* in 1929–30, 1931–33 and again in 1935–37 when he was principal scientist. It was in these years that Deacon provided the essential background to any understanding of the biology of whales, when he laid the foundations of modern knowledge about the water masses and water movements in the Southern Ocean.

During this period—again to introduce a personal note—those of us who served on the Fisheries Committee of the Development Commission (which one then hoped might evolve into a Fisheries Research Council) became increasingly concerned about the

uneven balance of British marine research, so disproportionately strong on the biological side. Something in the nature of an institute of physical oceanography was clearly needed. In an unpredictable manner this was to emerge from the War-time activities of George Deacon at the Admiralty Research Laboratory. In 1949 the staff of that laboratory united with the biologists from the *Discovery* investigations to form the National Institute of Oceanography, later, under the umbrella of the Natural Environment Research Council, to become the Institute of Oceanographic Sciences. In this way the scientific interests of the Admiralty became re-associated with the main stream of oceanographic research.

Little space is left to write about the contents of this impressive tribute, which begins with a list of Sir George Deacon's papers followed by 39 contributions from an even greater number of writers (as many have joint authorship). About half of these come from former colleagues at the Institute, the remainder from almost every leading centre of oceanographic research in the world. Their subject matter is no less diverse, a consequence of the immense range of modern oceanographic research. Articles dealing with the water characteristics of the Southern Ocean and much else concerning Antarctic water are followed by others covering many aspects of physical oceanography and then by contributions on biological subjects. The volume concludes, as appropriately as it begins, with reference to the whaling interests which have been of such momentous consequence in the development of British deep-sea research.

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Metal-ligand equilibria

Atlas of Metal-Ligand Equilibria in Aqueous Solution. By J. Kragten. Pp. 781. (Wiley: New York and London, 1978.) £35.

THE study of metal-ligand equilibria has often been assisted, following Ringbom, by the use of the parameter M' (the total concentration of metal ion not involved in the main reaction), the side-reaction coefficient (α , equal to $M'/(free M^{n+})$), and conditional constants. For those familiar with these concepts, this atlas provides in convenient graphical form the results of computer calculations for 45 commonly-used metals in the presence of 29 common ligands. These can be used for a variety of problems in aqueous

solution chemistry and particularly for the prediction of hydroxide precipitation and in the planning of analytical procedures.

The expert will query some of the entries, a number of the omissions (for example, the ligands iodide and NTA), and the choice of some of the stability constants. As the jacket suggests the graphs could also be of considerable value to a much wider readership but unfortunately the introduction, which sets out to explain the value of the graphs, is not clearly written and will not be easily understood by the non-expert. As a result, one feels that many who could profit from this compilation will fail to refer to it.

C. S. G. Phillips

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BASS, Hyman, CASSIDY, Phyllis J., and KOVATIC, Jerald (edited by). *Contributions to Algebra*. (A Collection of Papers Dedicated to Ellis Kolchin.) Pp.xvii + 424. ISBN-0-12-080550-2. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$39.50; £28.05.

CASTI, John L. *Dynamical Systems and Their Applications: Linear Theory*. (Mathematics in Science and Engineering: A Series of Monographs and Textbooks, Vol. 135.) Pp.xv + 240. ISBN-0-12-163450-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$19.50; £13.85.

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TICKER, Wallace H. *Radiation Processes in Astrophysics*. Pp.xi + 311. ISBN-0-262-70010-7. (Cambridge, Mass. and London: The MIT Press, 1977.) \$6.95.

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Physics

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BATTLE, G. C., CONNOLLY, Tom, and KEESSE, Anne M. (compiled by). *Laser Window and Mirror Materials*. (Solid State Physics Literature Guides, Vol. 9.) Pp.xi + 290. ISBN-0-306-68329-6. (New York, Washington and London: IFI/Plenum, 1977.) \$114.

CINDRO, Nikola (edited by). *Nuclear Molecular Phenomena*. (Proceedings of the International Conference on Resonances in Heavy Ion Reactions, Hvar, Adriatic Coast, Yugoslavia, May 30-June 3, 1977.) Pp.xvii + 432. ISBN-0-444-85116-X. (Amsterdam, New York and Oxford: North-Holland Publishing Company, 1978.) Dfl. 120; \$52.25.

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MAIN, Iain G. *Vibrations and Waves in Physics*. Pp.xiii + 336. ISBN-0-521-21662-1. (Cambridge, London and New York: Cambridge University Press, 1978.) Hardcover £17.50; Paperback £4.95.

PAO, Yoh-Han (edited by). *Optoacoustic Spectroscopy and Detection*. Pp.xi + 244. ISBN-0-12-544150-9. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$19; £13.50.

PERLMUTTER, Arnold, and SCOTT, Linda (edited by). *The Significance of Non-linearity in the Natural Sciences*. (Orbis Scientiae). (Studies in the Natural Sciences, Vol. 13.) Pp.viii + 423. ISBN-0-306-36913-3. (New York and London: Plenum Press, 1977.) \$51.

RENDLE, A. Barnard. *Gravitation and the Flowing Medium*. Pp.21. (Gt. Bookham, Surrey: Modern Research, 51 Dorking Road, 1978.) £1.

RIAZUDDIN (edited by). *Physics and Contemporary Needs*. Vol. 1. Pp.xii + 434. ISBN-0-306-31083-X. (New York and London: Plenum Press, 1977.) \$51.

SCHWARZ, Helmut, and HORA, Heinrich (edited by). *Laser Interaction and Related Plasma Phenomena*. Vol. 4A. Pp.lxxv + 1-602. ISBN-0-306-37144-8(4a). (New York and London: Plenum Press, 1977.) \$47.40.

SCHWARZ, Helmut J., and HORA, Heinrich (edited by). *Laser Interaction and Related Plasma Phenomena*. Vol. 4B. Pp.xvi + 603-1161. ISBN-0-306-37154-5 (v.4b). (New York and London: Plenum Press, 1977.) \$47.40.

TISZA, Laszlo. *Generalized Thermodynamics*. Pp.xii + 384. ISBN-0-262-70017-4. (Cambridge, Mass. and London: The MIT Press, 1977.) \$6.95.

TRICKEY, Samuel B., ADAMS, E. Dwight, and DUFFY, James W. (edited by). *Quantum Fluids and Solids*. Pp.x + 473. ISBN-0-306-31079-1. (New York and London: Plenum Press, 1977.) \$57.

VAVILOV, V. S., and UKHIN, N. A. *Radiation Effects in Semiconductors and Semiconductor Devices*. Translated from Russian. Pp.280. ISBN-0-306-10935-2. (New York and London: Consultants Bureau, 1977.) \$30.

ZANIO, Kenneth. *Semiconductors and Semimetals*. Vol. 13: *Cadmium Telluride*. (Semiconductors and Semimetals: A Treatise, Vol. 13.) Pp.xvi + 235. ISBN-0-12-752113-5 (v.13). (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$19.50; £13.85.

ZICHICH, Antonino (edited by). *New Phenomena in Subnuclear Physics*. Part A: Pp.xvii + 1-558. ISBN-0-306-38181-8 (part A). \$51. Part B: Pp.xi + 559-1243. ISBN-0-306-38182-6 (part B). \$59.40. (The Subnuclear Series, Vol. 13.) (New York and London: Plenum Press, 1977.)

Chemistry

BOCKRIS, J. O'M., and CONWAY, B. E. (edited by). *Modern Aspects of Electrochemistry*. No. 12. Pp.xii + 325. ISBN-0-306-37652-0. (New York and London: Plenum Press, 1977.) \$39.

COOK, D. B. *Structures and Approximations for Electrons in Molecules*. (Ellis Horwood Series in Chemical Science.) Pp.294. ISBN-85312-068-4. (Chichester: Ellis Horwood, Ltd., 1978. Distributed by Halsted Press, a Division of John Wiley and Sons, New York and London.) £13.50.

EYRING, Henry, and HENDERSON, Douglas (edited by). *Theoretical Chemistry: Advances and Perspectives*. Vol. 3. Pp.xi + 239. ISBN-0-12-681903-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$25; £17.75.

FRAZIER, Stephen E., and SISLER, Harry H. (edited by). *Chloramination Reactions*. (Benchmark Papers in Inorganic Chemistry/6.) Pp.xiii + 228. ISBN-0-87933-288-3. (Stroudsburg, Penn.: Dowden, Hutchinson and Ross, Inc., 1977. Distributed by Halsted Press, a Division of John Wiley and Sons, Inc., New York.) \$28.

GUNSTONE, F. D., SMITH, D. M., and SHARP, J. T. *An Introductory Course in Practical Organic Chemistry*. Pp.xv + 174. ISBN-0-412-21570-5. (London: Chapman and Hall, 1978. First published 1970.) £2.95.

HOW TO USE BEILSTEIN: *Beilstein Handbook of Organic Chemistry*. Pp.34. (Frankfurt/Main: Beilstein Institute, 1978. Distributed by Springer-Verlag, Berlin and New York.) gratis.

KLEMPNER, Daniel, and FRISCH, Kurt C. (edited by). *Polymer Alloys: Blends, Blocks, Grafts, and Interpenetrating Networks*. (Polymer Science and Technology, Vol. 10.) Pp.x + 491. ISBN-0-306-36410-7. (New York and London: Plenum Press, 1977.) \$57.

MACDONALD, Digby D. *Transient Techniques in Electrochemistry*. Pp.xii + 329. ISBN-0-306-31010-4. (New York and London: Plenum Press, 1977.) \$45.

announcements

Appointments

Professor O. L. Wade succeeds Professor E. Brodie Hughes as Dean of the Faculty of Medicine and Dentistry in the University of Birmingham for 5 years from 1 October.

Dr D. A. Ritchie from the Department of Virology, University of Glasgow, to the Chair of Genetics at the University of Liverpool.

Dr David Evered has been appointed Director of the Ciba Foundation from 1 September.

Professor Ian M. Allison has been appointed Professor and Head of the Department of Mechanical Engineering at the University of Surrey.

Dr Michael S. Halliday succeeds Professor Cohen to the Chair of Psychology in the University of Sussex from 1 October.

Dr D. M. Conning has been appointed Director of the British Industrial Biological Research Association as from 1 April.

Dr K. Boddy will become an honorary professor in medical physics at the University of Newcastle upon Tyne on 17 April, the date on which he takes up his appointment as head of the Regional Medical Physics Service.

Professor Ewan S. Page succeeds Dr H. R. Pitt as Vice-Chancellor of the University of Reading.

Dr Marvin L. Goldberger assumes his duties as president of the Californian Institute of Technology from 1 July.

Person to person

Exchange furnished flat or house for four people for period late July–August 1978, in Lisbon–Oeiras area, Portugal, for 3-bedroom house in Birmingham for the same period. Contact C. C. Thornburn, Department of Biological Sciences, Aston University, Birmingham, UK.

Exchange 3-bedroom fully furnished centrally-heated home near London for similar in Los Angeles convenient for USC and UCLA, for 9 months or one year from late August 1978. London house 35–40 minutes from University College, British Museum, L.S.E. Contact E. A. Power, Department of Mathematics, University College, Gower Street, London WC1, UK.

Dr K. G. Stephens has been appointed to a personal Chair in Electrical Engineering at Surrey University.

Meetings

9 May, **International Symposium on Crop Protection**, Gent (Rijksuniversiteit Geny Faculteit van de Landbouwwetenschappen, B-9000 Gent Belgium). 15–19 May, **3rd International Conference on Basement Tectonics**, Colorado (Basement Tectonics Committee, Fort Lewis College, Colorado 81301).

2–8 July, **12th Meeting of the Federation of European Biochemical Societies**, Dresden (12, FEBS-Meeting, Dresden, PO Box 313, GDR).

3–13 July, **Symposium on Mathematical Theory of Non-Linear Problems in Quantum Mechanics and Quantum Optics**, Durham (E. A. Power, Mathematics Department, UCL, Gower Street, London WC1, UK).

5–8 July, **New Perspectives on Antipsychotic Drug Action**, Beerse (Josée Leysen, Department of Biochemical Pharmacology, Janssen Pharmaceutica, B-2340 Beerse, Belgium).

10–14 July, **3rd International Symposium on Cytopharmacology**, Venice (B. Ceccarelli, C.N.R. Center of Cytopharmacology, Institute of Pharmacology of Milan, Via Vanvitelli 32, 20129 Milan, Italy).

10–14 July, **Micro 78**, London (Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

10–15 July, **International Abscission Conference**, Gainesville, Florida (C. W. Cooper, US Horticultural Research Laboratory, Orlando, Florida 32803).

16–20 July, **4th American Conference on Crystal Growth**, Washington (Dr Robert L. Parker, Materials Building, Room B164, National Bureau of Standards, Washington D.C. 20234).

16–21 July, **7th International Congress of Pharmacology**, Paris (Professor Paul Lechat, 1 Rue Jules Lefebvre, 75009, Paris, France).

17–22 July, **25th International Field Emission Symposium**, Albuquerque (J. A. Panitz, Sandia Laboratories, Surface Physics Division 5114, Albuquerque, New Mexico 87115).

23–25 July, **Pharmacological Modulation of Steroid Action**, Turin (Fondazione Giovanni Lorenzini, Via Monte Napoleone 23, 20121 Milan, Italy).

23–27 July, **International Conference on Fish Science and Technology**, Aberdeen (Torry Research Station, 135 Abbey Road, Aberdeen, UK).

Reports & Publications

Other countries—February

Nestlé Research News 1976/77. Pp.148. (Lausanne, Switzerland: Nestlé Products Technical Assistance Co., Ltd. Technical Documentation Center, 1977.) [152]

Annali dell'Istituto Sperimentale per la Elaiotecnica, Volume V, 1975. Pp.276. (Pescara, Italia: Istituto Sperimentale per la Elaiotecnica, 1975.) [162]

National Council on Radiation Protection and Measurements. NCRP Report No. 56: Radiation Exposure from Consumer Products and Miscellaneous Sources. Pp.80. (Washington, DC: National Council on Radiation Protection and Measurements, 7910 Woodmont Avenue, 1977.) [162]

Bulletin of the American Museum of Natural History, Vol. 159, Article 3: Observations on Intertidal Organism Associations of St. Catherine's Island, Georgia. I. General Description and Paleoeological Implications. By Robert W. Morris and Harold B. Rollins. Pp.87–128. (New York: American Museum of Natural History 1977.) \$2.80. [162]

Nuclear Sweden III. Pp.31. (Stockholm: Swedish Atomic Forum, Sifo, Box 5506, 1978.) [212]

Chondrogenesis of the Somitic Mesoderm. By Brian Keith Hall. (Advances in Anatomy, Embryology and Cell Biology, Vol. 53, Fasc. 4.) Pp.50. (Berlin and New York: Springer-Verlag, 1977.) DM26; \$12. [212]

Fisheries and Environment Canada. Fisheries and Marine Service Technical Report No. 745: Some Effects of Potato Farming and Forest Clearcutting on New Brunswick Streams. By H. E. Welch, P. E. K. Symons and D. W. Narver. Pp.iv+13. (St. Andrews, NB: Biological Station, Fisheries and Marine Service, 1977.) [212]

US Department of the Interior: Geological Survey. Professional Paper 1027: Paleozoic-Mesozoic Boundary in the Berry Creek Quadrangle, Northwestern Sierra Nevada, California. By Anna Hietanen. Pp.iii+22. (Washington, DC: US Government Printing Office, 1977.) [212]

The Australian Mineral Development Laboratories. Seventeenth Annual Report, 1977. Pp.36. (Frewville, South Australia: AMDEL, 1978.) [212]

State of Israel. Ministry of the Interior: Environmental Protection Service. Selected Papers on the Environment in Israel, No. 5. Edited by Naomi Ragen. Pp.155. Israel in the Mediterranean Ecosystem. (Israel National Report to The Conference of the Coastal States of the Mediterranean Region for the Protection of the Mediterranean Sea, Monaco, 9–14 January 1978.) By David Greenberg and Richard A. Hellman. Pp.70. (Jerusalem: Ministry of the Interior, Environmental Protection Service, 1977 and 1978.) [212]

Annals of the South African Museum. Vol. 73, Part 10: A New Species of *Halicyclops* (Copepoda, Cyclopoida) from Estuaries in Transkei, Southern Africa. By T. Wooldridge. Pp.361–371. R.1.80. Vol. 74, Part 1: Cretaceous Faunas from Zululand and Natal, South Africa. *A. Joubertianus* from the Mzime Formation (Albian). By William James Kennedy and Herbert Christian Klinger. Pp.1–12. R.1.90. Vol. 74, Part 3: Illustrated Generic Key to South African Continental Ostracoda. By W. G. McKenzie. Pp.45–103. R.4.10. Vol. 74, Part 4: The South African Museum's *Meiring Naude* Cruises. Part 6: Amphipoda. By Charles Griffiths. Pp.105–123. R.5.20. (Cape Town: South African Museum, 1977.) [222]

Bulletin of the American Museum of Natural History, Vol. 159, Article 2: Revision and Phylogeny of *Zalobius*, *Asemobius*, and *Nanobius*, New Genus (Coleoptera: Staphylinidae, Piestinae). By Lee H. Herman, Jr. Pp.45–86. (New York: American Museum of Natural History, 1977.) \$2.70. [222]

Swedish College of Forestry. Studia Forestalia Suecica. Nr.140: Effects of Nitrogen Fertilization on the Abundance of Enchytraeids and Microarthropods in Scots Pine Forests. By Eirik Lohm, Helene Lundkvist, Trygve Persson and Anders Wären. Pp.23. Nr.141: Effects of Spacing and Fertilization on Four Grafted Clones of Scots Pine. By H. H. Hattemer, E. Andersson and C. O. Tamm. Pp.31. Nr.142: External Factors Influencing Female Flowering in *Picea abies* (L.) Karst. By Katarina Lindgren, Inger Ekberg and Gösta Eriksson. Pp.53. Nr.143: The Swedish Roundwood Market—Parties Active on the Market—Wood Price Negotiations. By Lars Lönnstedt. Pp.55. (Stockholm: Swedish College of Forestry, 1977.) [232]

Republic of South Africa: Department of Industries, Sea Fisheries Branch Investigational Report, No. 114: Influence of Temperature, Dissolved Oxygen and Salinity on Incubation and Early Larval Development of the South West African Pilchard *Sardinia ocellata*. By D. P. F. King. Pp.35. (Cape Town: Department of Industries, Sea Fisheries Branch, 1977.) [272]

Mauritius Sugar Industry Research Institute. Annual Report for 1976. Pp.84. (Reduit, Mauritius: Mauritius Sugar Industry Research Institute, 1977.) [272]

Pour Ube Conception Quadrimensionnelle de la Détermination Par José Marie. Pp.132. Théorie du Champ Pour un Univers Unitaire (I). Par José Marie. Pp.15. (Baziège: José Marie, 19, rue de l'Espinet, 1978.) [272]

UK & Ireland — March

- Seed Production Techniques in West Germany and The Netherlands. By P. A. Dover. (A Report of the Study Tour Undertaken from 25 June to 8 July 1977.) Pp. 46. (London: Potato Marketing Board, 50 Hans Crescent, SW1, 1977.) [13]
- Planning and Plutonium: Evidence of the Town and Country Planning Association to the Public Inquiry into an Oxide Reprocessing Plant at Windscale. Pp. xviii+110. (London: Town and Country Planning Association, 17 Carlton House Terrace, SW1, 1978.) £2. [33]
- Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Fisheries Research Technical Reports. No. 38: MAFF Current Meter Data Inventory 1975-76. By K. J. Medler. Pp. 28. No. 40: Paralytic Shellfish Poisoning—An Account of Investigation into Mussel Toxicity in England 1968-77. By P. A. Ayres and Mary Cullum. Pp. 23. No. 41: Population Dynamics of the Lobster *Homarus gammarus* (L.) Off the Coasts of England. By B. T. Hepper. Pp. 29. No. 42: Studies on the Indirect Effects of Fishing on Stocks of Cockles, *Cardium edule*, in the Thames Estuary and Wash. By A. Franklin and G. D. Pickett. Pp. 9. No. 43: A Survey of the Dispersal of Colliery Waste from Lynemouth Beach, Northumberland. By R. S. Nunny. Pp. 17. (Lowestoft, Suffolk: Directorate of Fisheries Research, Fisheries Laboratory, Pakefield, 1977 and 1978.) [33]
- Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 282. No. 990: The English Silurian Fossil *Placocystites forbesianus* and the Ancestry of the Vertebrates. By R. P. S. Jefferies and D. N. Lewis. Pp. 205-323 + plates 1-15. (London: The Royal Society, 6 Carlton House Terrace, SW1, 1978.) UK £11; Overseas £11.50. [33]
- High Pressure Technology Association. High Pressure Safety Code. Pp. 46. (London: High Pressure Technology Association, c/o Dr G. Saville, Dept. of Chemical Engineering, Imperial College, SW7, 1977.) £2; \$6. [63]
- Proceedings of the Royal Irish Academy. Section A: Mathematical and Physical Sciences. Vol. 77. A, No. 8: Residual Properties of Groups Determined by Ideals. By T. C. Hurley. Pp. 97-104. (Dublin: Royal Irish Academy, 1977.) £1.15. [63]
- Ministry of Overseas Development. Overseas Research Publication No. 25: British Overseas Aid—Agricultural Research (Crop and Soil Sciences), 1968-1973. Edited by L. Kasasian, R. K. Cunningham, R. W. Smith and W. D. Brind. Pp. 85. (London: HMSO, 1978.) £4.50 net. [63]
- The Hannah Research Institute for Studies Relating to the Production and Utilization of Milk—Report for 1977. (University of Glasgow.) Pp. 70. (Ayr: The Hannah Research Institute, 1978.) £1.50. [73]
- Health and Safety Executive. Health and Safety: Agriculture. Pp. 18. (London: HMSO, 1978.) £1. [73]
- Publications Review: Management—Technology—Policy. Vol. 1, No. 1, January 1978. Editor-in-Chief: Cyril C. Gee. Pp. 1-28. Annual Subscription (4 issues): UK £25; All other countries US\$60 or foreign equivalent. (London: NPM Infotink Ltd., 5 Stone Buildings, Lincoln's Inn, WC2, 1978.) [83]
- Imperial College of Science and Technology. (University of London.) Research Report, 1974-78. Pp. 281. (London: Imperial College of Science and Technology, 1978.) [83]
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